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SOME NUTRITIONAL ASPECTS IN MOTTLE-LEAF AND OTHER PHYSIOLOGICAL DISEASES OF CITRUS[1]

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INTRODUCTION

One of the chief purposes in the growing of citrus in artificial cultures is to help to interpret the growth of citrus in the field. The factors involved in the field are often too complex or too numerous to allow cause and effect to be distinguished, and even in artificial cultures with soil the results may not be easy to interpret.

The growth of citrus in sand or solution cultures, while frequently reducing the number of factors involved, requires first of all a knowledge of how to grow healthy citrus over a period of years instead of days or weeks. There is as yet no reproducible standard citrus plant for a given environment. Through the shortcomings in efforts to adjust the environment to obtain healthy growth, knowledge is frequently acquired concerning physiological disease.

The physiology of citrus is far from being understood. It is believed that the system that connects the leaves with the rootlets and soil is very complex. The intermediate root, trunk, and twigs have important functions interrelated with both extremities of the tree. In the present paper attention has been given mainly to the producing of symptoms, without stressing the complexity of the systems involved. It is hoped that further difficulties in the growing of healthy citrus under controlled conditions over long periods of time will add stimulus in overcoming and eventually understanding the difficulties.

Some of the more important physiological diseases of citrus are mottle-leaf, chlorosis, salt or tipburn, deficiency effects, and many others. Mottle-leaf is by far the most important of these and perhaps the most baffling in its causes and control. Among the many possible explanations of mottle-leaf in citrus are an inadequate supply of nitrogen, an excess of nitrogen, lack of humus, deficiencies of certain elements, toxic minerals in soils and irrigation water, and organic soil toxins. Some of these nutritional aspects in physiological diseases of citrus form the basis of the present paper.

METHOD OF GROWING CITRUS CULTURES

Solutions Employed.—In solution cultures with cuttings the solution employed by Hoagland has been, with slight modification, extensively used in these experiments. Stock solution A consists of 133.3 grams KNO_3, 200 grams $MgSO_4 \cdot 7\ H_2O$, and 6.1 grams NaCl in 2,000 cc distilled water; stock solution B consists of 288.8 grams

$Ca(No_3)_2 \cdot 4H_2O$ in 2,000 cc distilled water; and stock solution C consists of 100 grams KH_2PO_4 in 2,000 cc distilled water. Hoagland's culture solution is prepared as follows: 99 cc of stock solution A, 117 cc of stock solution B, and 54 cc of stock solution C are brought to a volume of 18 liters with distilled water. In the experiments reported in this paper 234 cc of stock solution B was used in most cases. A minimum amount of a weak solution of freshly prepared iron tartrate was added to the distilled water used. A concentration of 0.1 p.p.m. of boron as boric acid was used in the culture solution to avoid certain symptoms of boron deficiency described by Haas[12] and Haas and Klotz.[15, 16]

Aeration of Solutions.—When cuttings are grown in 2-quart Mason jars for several months without frequent change of solution, the roots are largely confined to the upper half of the jar, presumably as a consequence of the CO_2—O_2 relation at the top where the distilled water is added. Such jars therefore were used only for seedlings having few roots and for experiments of short duration. Zimmerman[45] has shown a close relation to exist between the oxygen supply in culture solutions and root growth.

After removal from the sand of the rooting chamber leafy cuttings were first established in 2-quart Mason jars. From these jars the cuttings were transferred to shallow, white enamel-ware pans (Vollrath 8½-qt., 136-solution bowl, about 10 inches in diameter at the bottom, 14 inches at the top, and about 5 inches deep) or to small bath tubs of about 9-liter capacity (Old Colonial Porcelain Steel Sanitary Ware). Distilled water containing iron tartrate was run into each pan to restore the original volume daily except Sundays and holidays. The large volume of water thus frequently added supplied adequate oxygen for root growth, the shallowness of the pan insuring aeration throughout the culture. It was found that under these conditions it was unnecessary to renew the culture solution for several months.

The technique followed by investigators in obtaining the best root growth varies almost with each investigator. In some cases the culture solutions are aerated directly; in others only the tip of the tap roots is kept in the shallow solution, while the upper portion of the root system is in a humid atmosphere or receives a fine mist by the blowing of air into the shallow solution; and in still other cases the solutions are continuously renewed or at intervals.

Desiccation of Roots.—Even when the culture pans are covered with planed Texas pine boards that have been coated several times

with shellac and then with Valspar, the atmosphere above the surface of the solution is not sufficiently moist to prevent injury to the root system. The upper portions of the roots are only slightly, if at all,

Fig. 1. One of many lemon cuttings grown more than a year in shallow enamelware pans of culture solution containing 5 p.p.m. of zinc and 0.1 p.p.m. of boron.

corked over, and gradually, if the solution has been allowed to fall at times to about two-thirds of the original volume, this upper portion of the root system begins to deteriorate. Gradually the formerly healthy

white root tips in the lower portion of the root system take on a brownish aspect and later appear gelatinous. In this case injury to the root system begins from above and proceeds downward, quite the opposite of that occurring in 2-quart Mason culture jars in which the rootlets in the nonaerated solution in the lower half of the jar are the first to deteriorate.

It is not always apparent from an observation of the tops of citrus cuttings many feet in height that the root system is gelatinous and in a rotted condition. In fact the rotted portion of the root system in a solution culture may be cut away, leaving only stubs of the original roots, and the foliage of such plants, under glasshouse conditions, does not wilt. Judging solely by the appearance of the tops of such cultures one would be quite unaware of the poor condition of the root system. A new equilibrium is reached by a gradual abscission of the oldest leaves.

To prevent the desiccation of the upper portion of the root system the solution was maintained at a sufficiently high level by means of the apparatus shown in figure 1. This consisted of a gallon bottle and a leveling tube containing a dilute solution of iron tartrate which was automatically delivered as the surface was lowered by transpiration below the end of the delivery tube. Such an arrangement also enables one to measure the amount of water transpired.

The injury described as resulting from the drying of roots is of considerable interest, although it is very little understood. The effects of desiccation are well known, however, in the case of citrus seeds. These are shipped in or kept in moist sphagnum after they have been removed from the fruit pulp; otherwise germination is impaired. In this case the bad effects may involve a destruction of hydration power of the seed coats. No data are now available regarding the amount of pectic substances in citrus roots or other portions of the tree except the fruit. It is not unlikely that pectin occurs more widely distributed throughout the citrus tree than is commonly assumed, for it has already been described by Tutin[(41)] as occurring in apple leaves.

The injury described as resulting from the desiccation of roots may also be kept in mind when soil is permitted to dry out. By making trenches in citrus orchards grown on very sandy soils it was found that many rootlets in close proximity to the surface of the soil were injured or had died from the lack of moisture between irrigations. There may be a considerable difference in the effects of desiccation upon the tree according to whether or not the roots affected are the young absorbing ones or those that have undergone more complete maturity transformations.

ZINC IN CULTURE SOLUTION

Beneficial Effect.—Unusually healthy growth of the roots was obtained when concentrations not exceeding 5 p.p.m. of zinc (according to the size of the plant) were added to the culture solution in the form of $Zn(NO_3)_2 \cdot 6\ H_2O$ and the original volume continuously maintained. In this case the roots maintained a vigorous white appearance, and the organic residues resulting from the collection of dying rootlets in the bottom of the pans were at a minimum.

Fig. 2. Lemon cutting grown in a shallow enamel-ware pan of culture solution containing boron and zinc. The root system is in an excellent state of health after more than a year's growth.

Zinc appeared to improve the quality of the growth but not the quantity. Figure 2 shows a lemon cutting from a solution culture that received both boron and zinc and indicates the unusually healthy growth. Where the cultures of citrus were maintained for periods of several years the addition of zinc was necessary to maintain health. Many of the citrus cuttings supplied with zinc were heavily laden with fruit.

Sommer and Lipman[40] and Sommer[39] have found zinc and boron indispensable for the growth of higher green plants. McHargue[28] has found a considerable range of concentration of zinc in soils, plants, and animals. Some beneficial effect of zinc was observed by Allison, Bryan, and Hunter[1] on the raw peat soils of the Florida Everglades, although the mode of action is not clear. In sand cultures where the roots have penetrated the asphalt paint of the zinc-coated containers and come in contact with the zinc, the plants have grown remarkably well.

In the case of citrus cuttings in culture solution the amount of zinc required at any one time may be extremely small, since much of the zinc is precipitated as sulfate or phosphate in the culture solution. Even if the zinc promotes better root growth and later analyses should prove that it has been absorbed, it is still possible that the benefits obtained with zinc may not be the result of satisfying a zinc deficiency but rather that of a disinfectant action of the zinc in the culture solution. However, no other heavy metal in small concentrations has thus far been found to bring about the same beneficial response from the root system over a period of months or years. Thus far the writer has not used copper in the culture solution without finding it appreciably toxic. This is in agreement with the results of Sommer and Lipman.[40] It is very likely that beneficial concentrations of salts of such metals may be found to occur in the range of the most extremely minute amounts.

It was found that concentrations up to 5 p.p.m. of zinc in the culture solution are suitable for large cuttings that are making considerable new growth but are injurious to young cuttings or seedlings that do not make much growth. For such smaller plants the concentration of zinc should be greatly reduced; in fact in these experiments zinc was frequently omitted until the cutting had grown large enough to warrant its transfer from the 2-quart Mason jar to the pan, since its use seems to be most helpful after months of growth. Likewise boron has frequently been omitted until the absence of new growth and the presence of corky veins indicated its need. The time interval before boron became a limiting factor depended upon the nature and solubility of the culture vessel and the size of the cutting.

Injurious Effect.—In order to determine the toxic action of zinc when it is present largely in a precipitated form in the culture solution, Russet lemon seedlings were grown in 2-quart Mason jars of culture solution containing concentrations of 0.0, 2.5, 5.0, 7.5, 10.0, 15.0, and 20.0 p.p.m. of zinc as zinc nitrate. The seedlings were

germinated in moss, transferred to the jars on July 19, and grown until the following November 20. The concentrations were in triplicate and each jar contained five seedlings. The culture solutions containing the zinc were renewed at intervals of a few weeks.

Figure 3 shows the type of growth made in the cultures containing different concentrations of zinc. At 5 p.p.m. the zinc was injurious

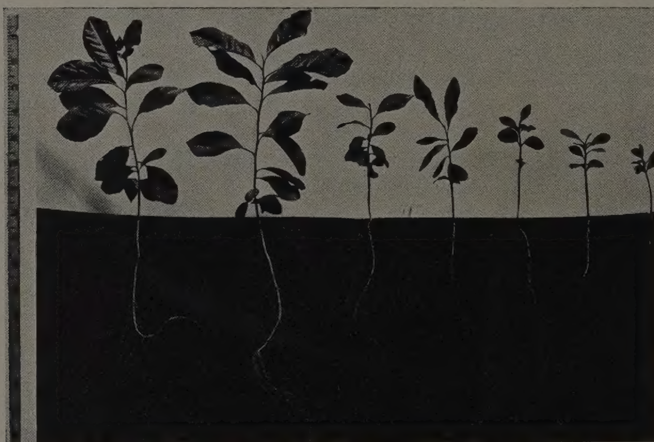

Fig. 3. The toxic action of zinc in culture solution upon the growth of Russet lemon seedlings in 2-quart Mason jars. At 5 p.p.m. or higher concentrations of zinc, the leaves are small and the internodes are shortened.

under the conditions of the experiment; at this and higher concentrations the roots were injured and the interval between the leaves on the stem axis was shortened, giving some seedlings a dwarfed or rosette-like appearance. A considerable amount of the zinc that is added to the culture solution may become insoluble during the period of the experiment. However, as with supposedly insoluble boron compounds, the minute amount that is soluble at any one time is removed by the roots and the solubility and absorption may be progressive to the point of injury.

EFFECT OF NONGASEOUS ELEMENTS OF LOWEST ATOMIC WEIGHT IN GROUPS I, II, AND III IN THE PERIODIC SYSTEM

Lithium and Boron.—The toxic action of lithium and of boron upon citrus has previously been described (Haas[10, 11]). Lithium has been found in several counties of southern California near but not as yet in soils planted to citrus. Several years ago some soil cultures with budded Valencia orange trees received large applications of potassium nitrate and were then injured by the application of boron to the soil. The mature leaves of these trees at the present time still show distinct boron injury, even though the soil has been leached many times at various intervals. It indicates that in some soils at least boron injury may persist for a long period of years, especially as the leaves attain full maturity.

Beryllium.—The relation of beryllium to lithium and boron in the periodic system, together with the well-known fact that it occurs widely distributed in the earth's crust (Petar[32]) led the author to test the effect of this element upon citrus. Because of the presence of unidentified beryl (Hillebrand[19]) there is little doubt that in many of the chemical analyses of soils the beryllium is included in the values given for aluminum. Parsons and Barnes,[31] Kolthoff,[23] Dixon,[4] Fischer,[5] and others have described methods for the separation and estimation of beryllium. Titanium, iron, aluminum, and boron may interfere.

Since no studies to test the effects of beryllium on citrus have previously been made either with solution cultures or in the field, the following experiments should be of interest. Many of these experiments were carried on with citrus seedlings in Hoagland's solution in 2-quart Mason jars for periods of three months. Eureka lemon, rough lemon, rough orange, sweet orange, and grapefruit seedlings were used. The Eureka lemons used as the source for seed with which to grow the seedlings were not obtained from variegated lemon trees or from unknown lots of lemons, but in each experiment were selected in the field as being typical fruit of healthy commercial Eureka lemon trees. No boron or zinc was added. The concentration of beryllium, added as beryllium nitrate, ranged from 0.0 to 25.0 p.p.m. with 2.5 p.p.m. intervals. At a concentration of 2.5 p.p.m. of beryllium, rough lemon seedlings were only slightly affected, and then

only the lowermost or oldest leaves. At 5.0 p.p.m. and higher there was a decrease in the growth of the tops and increasing injury to the oldest leaves. With Eureka lemon seedlings, the oldest leaves showed effects at 2.5 p.p.m. and increasing injury with increasing concentration. Rough orange, sweet orange, and grapefruit seedlings were apparently unaffected at 2.5 p.p.m., but at 5.0 p.p.m. the toxic effect became evident.

In another experiment with rough lemon seedlings grown for nine months in solution cultures containing concentrations of beryllium

Fig. 4. Effect of toxic concentrations of beryllium on leaves of lemon seedlings in solution cultures. Normal healthy leaf of control cultures to the left in upper row.

ranging from 0.0 to 10.0 p.p.m. at about 0.5 p.p.m. intervals, definite toxic effects were produced with about 2.0 p.p.m. of beryllium. The effects produced in the leaves of lemon seedlings were distinct from those in orange or grapefruit, in which the leaves turned orange or golden yellow, but not without severe burning. The beryllium-affected leaves of lemon seedlings are shown in figure 4. The yellow areas throughout the leaves give them the appearance of variegation. At the higher concentrations of beryllium small burned spots become evident.

It was thought that seedlings might show symptoms or effects of a different type than larger plants such as cuttings. Through the kindness of Dr. F. F. Halma many Washington Navel orange, Valencia orange, grapefruit, and Eureka lemon cuttings were obtained which

had been rooted in sand. These were grown in solution cultures in pans for varying periods and were then subjected to different concentrations of beryllium in Hoagland's solution to which 5.0 p.p.m. of zinc, but no boron, was added. The new pans (Vollrath 8½-qt., 136-solution bowl) supplied some boron, and no boron deficiency symptoms were evident throughout the duration of the experiments.

Twelve large lemon cuttings one or more years of age were used in one experiment that lasted from December 3, 1929, to September 3, 1930. The concentrations of beryllium used were 0.00, 2.73, 5.46, 8.19,

Fig. 5. A type of mottling produced in leaves of lemon cuttings grown in solution cultures containing beryllium added as beryllium nitrate.

10.92, and 13.65 p.p.m., and were in duplicate. At the end of the experimental period the cuttings in the control cultures and in the 2.73 p.p.m. concentration of beryllium were in good condition. Above this concentration of beryllium the roots showed injury and there was considerable abscission of leaves. Some of the leaves appeared mottled (fig. 5). The veins remained green, but there was a type of mottle that extended throughout the length of the leaf blade, followed by some leaf burning on the ventral surface at the higher concentrations of beryllium. This type of mottling differs from chlorosis, in which only the veins remain a light green and the remainder of the leaf becomes yellowish green. Some of the lemon leaves in the cultures at 8.19 and 13.65 p.p.m. of beryllium showed the peculiar variegation or mottle seen in figure 6. The numerous minute yellow areas present a new type of mottling that has not yet been found in the field. It

appears that the effects of beryllium are quite distinct from those of boron or lithium. This is of interest in view of the low atomic weights of the three elements, their toxic properties, and the fact that a concentration of 0.1 p.p.m. or less of boron is necessary for healthy growth in citrus.

Two young cuttings each of Washington Navel orange, Valencia orange, grapefruit, and lemon that had been only a month or so in

Fig. 6. A type of mottling showing minute yellow areas in leaves of lemon cuttings grown in solution cultures containing beryllium.

pan cultures and had just started vigorous growth were subjected to the same concentrations of beryllium as in the former experiment, making in all 48 cultures of young, vigorous cuttings. After a few weeks in the culture solutions only the controls and the cuttings at the lowest concentration of beryllium appeared to be in good condition, as was also the case when young lemon cuttings were grown in similar solutions in 2-quart Mason jars. The remainder all showed

such rotting of roots and loss of leaves that an effort was made to induce recovery by replacing the toxic solution by the control solution. The injury, however, was found irreparable; this indicates that young cuttings are much more sensitive than older cuttings to the toxic action of such elements.

MOTTLE AND OTHER CHLOROPHYLL CHANGES INDUCED BY PICRIC ACID

It has been shown that a toxic agent such as beryllium may bring about a mottle of citrus leaves. Very little is known regarding the relation of the leaf pigments to one another even though a mottle involves chlorophyll changes. If root absorption of an oxidizing or reducing agent could be brought about it might be possible to produce a mottle by interference with chlorophyll transformations. The mottle in citrus leaves is of a yellow color. Therefore picric acid, which is both yellow dye and an oxidizing agent, was chosen for the experiments. Preliminary trials showed that none of a large number of red or blue dyes when placed in culture solutions containing citrus cuttings had any effect upon the leaves.

When picric acid is added in 5- or 10-gram amounts to 12-gallon earthenware containers of Sierra loam soil containing budded lemon trees, within a few days the leaves take on a brownish-green hue that is very easily recognized when compared with control trees. Figure 7 at the left, upper row, shows a healthy lemon leaf of a control tree; at the right, upper row, a yellow leaf ready to fall from a lemon tree grown in a water-logged soil culture; and in the middle, upper row, is a lemon leaf the color of which has been changed by the addition of picric acid to the soil. These leaves do not absciss for long periods unless too high an amount of the acid has been added, in which case tipburn results. After the soil was leached with distilled water the new growth from such shoots was normal.

Varying amounts of picric acid added to culture solutions of rough lemon seedlings reveal concentrations at which the picric acid changes the green chlorophyll to a brown or brownish-green color in certain small areas, as seen in the lower row of figure 7 (control leaf at the right). At high concentrations the leaves are burned. Two grams of picric acid was dissolved in 18 liters of distilled water. One hundred, 250, 500, 750, and 1,000 cc of this picric acid solution with enough culture solution to make 1,000 cc volume were used. In the solution containing only picric acid the leaves burned, while in those containing

500 and 750 cc of the acid the leaves took on the appearance shown in figure 7. When such discoloration occurs, growth is slowed up but the roots show no effect. If the concentration of picric acid in the solution is reduced, normal growth proceeds. This effect of picric acid in solution culture was obtained without the previous cutting off of any of the rootlets.

Fig. 7. Effect of picric acid on leaves: upper row, lemon leaves from soil cultures; left, control; middle, leaf colored a brownish-green by applying picric acid to soil; right, leaf ready to absciss from culture kept too wet (note pale veins). Lower row, leaves of rough lemon seedlings from solution cultures; extreme right, control; left of control leaf are leaves with brownish-green or brown areas as a result of the addition of picric acid to the culture solution.

If the culture solution containing picric acid is sampled the solution is still found to contain nitrate. The discoloration in the leaves cannot be due to a lack of sufficient nitrate resulting from the reduction of nitrate by the picric acid. It would appear either that the dye has been absorbed by the leaf in amounts sufficient to mask the color of the chlorophyll, in which case the proteins of the plastids possibly form yellow addition compounds, or has reacted with the chlorophyll causing interference in the oxidation-reduction process; or that the products of the action of the picric acid on nitrogen compounds in the leaf are toxic.

MOTTLE-LEAF IN RELATION TO NITROGEN DEFICIENCY OR EXCESS

Urea.—From the field there have been cases brought to the writer's attention in which a mottling or yellowing developed at the tips of orange leaves when certain compounds of urea were accidentally applied in excess to the soil about the last tree in each row. This type

Fig. 8. Orange leaves showing injury resembling mottle-leaf as a consequence of the accidental addition of an excess of calurea to the soil about certain trees.

of injury is shown in figure 8. In some instances the effects bore certain resemblances to boron injury although in most leaves they were distinct from it. Analysis of the fertilizer showed no boron. An effort was made to reproduce this condition in soil cultures. Preliminary experiments with young budded Valencia orange trees in

soil cultures have shown a similar type of mottle when high concentrations of calurea were applied. These experiments are being carried on further.

Figure 9 shows lemon leaves with yellowish-brown tips which were brought to the writer from groves that had received large applications

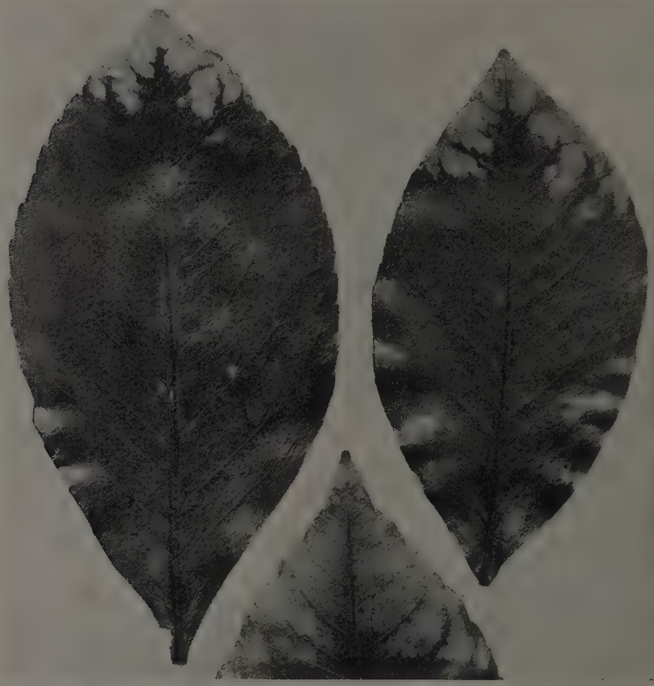

Fig. 9. Lemon leaves with yellowish-brown leaf tips after the application of urea-rich materials in the field.

of urea-containing fertilizers. The exact nature of this injury is not fully understood, although in soil cultures the application of ammonium carbonate with ammonium nitrate to the soil produced effects on lemon leaves very similar to those shown in figure 9. This is the more plausible when it is considered that urea is first converted into ammonium carbonate before the nitrification process.

Experiments carried on in water cultures with large citrus cuttings of several varieties showed that concentrations of 500 or 1,000 p.p.m.

of urea were extremely injurious within a few days. The injury was not a result of an excessive salt concentration. No injury results when double such concentrations of nitrate are used.

Ammonia.—Lipman[25] has theorized regarding the poor nitrifying power of soils as a possible cause not only of exanthema in lemons but also of mottle-leaf. Normal ammonifying power of soil coupled with poor nitrifying power is supposed to result in enforced ammonia absorption in the absence of nitrates, hence there is nitrogen hunger. Preliminary studies on orange trees in soil cultures which receive daily applications of solutions of weak ammonia show that at first

Fig. 10. Valencia orange leaves from trees grown about two years in nitrogen-deficient soil which was then given HNO_3 in distilled water. The leaves were yellow before the HNO_3 was added.

the growth is rapid as the nitrates are carried down to the roots, but that the enforced ammonia absorption soon causes the leaves to appear yellow as though in need of nitrate. The pH of a 1:2 soil suspension was 8.25, so that excessive alkalinity is not present. Citrus does better, however, at lower pH values. In the field the leaves of exanthema trees are as a rule unusually green and do not indicate nitrate hunger; in fact applications of large amounts of nitrate have not only been found to be ineffective but may increase the trouble.

Nitric Acid with Young Budded Citrus Trees.—Valencia orange trees were grown in Sierra loam in six 12-gallon earthenware containers. The cultures were given distilled water only until growth had ceased and the leaves were of a uniform yellow color. A total of 30 cc of concentrated HNO_3 in distilled water was then added to the soil in each container. In the course of a few days the leaves appeared as in figure 10. In some cases half of the leaf blade, with the midrib

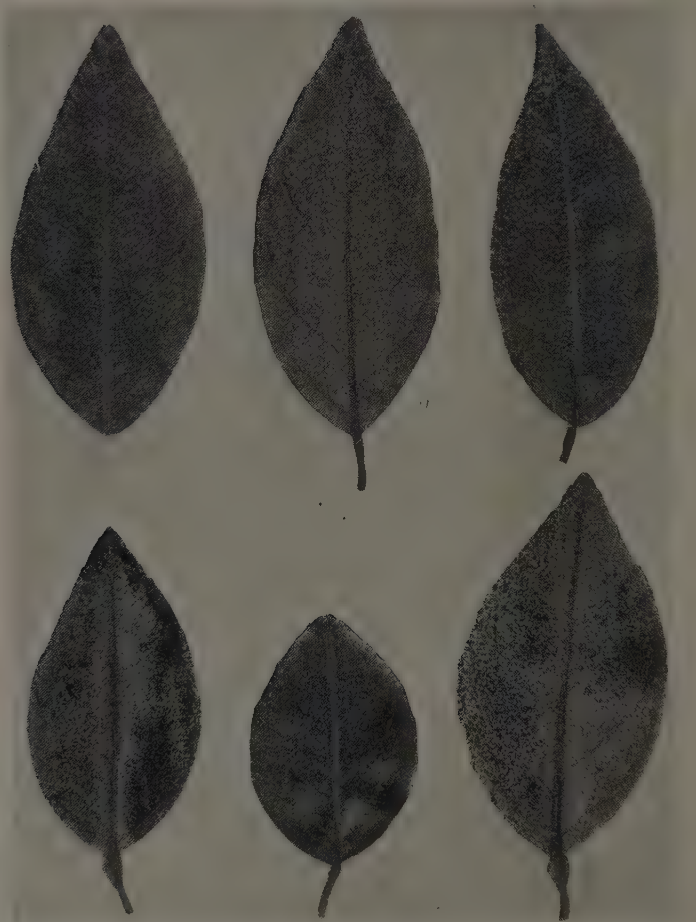

Fig. 11. Valencia orange leaves from trees in containers of soil: upper row, center leaf ventral side, other two leaves dorsal side, showing dry, corky sunken spots resulting from over-strong nicotine sulfate spray on immature leaves. Lower row: center leaf, dorsal side of nitrogen-deficient leaf after being made green by the application of HNO_3 to the soil, other two leaves, ventral side of greened-up leaves, showing numerous small resinous spots.

as the division line, became dark green while the remainder changed but little. At no time, either when the leaves were deficient in nitrogen or when an abundance of nitrate was available, did the coloring resemble that of mottle-leaf.

Although the application of HNO_3 caused the leaves to become dark green, their ventral surface was usually covered with gum spots. The center leaf in the lower row of figure 11 shows the dorsal surface while the other two leaves in the same row show their ventral surfaces covered with numerous gum spots. The dry, sunken corky spots on the leaves of healthy vigorous Valencia orange trees grown in rich soil are shown in the upper row of leaves in figure 11. The center leaf shows the ventral side, the others the dorsal side. These spots were produced by the use of an overstrong solution of a certain nicotine sulfate spray preparation on leaves that were only partially grown.

When citrus leaves are not allowed to reach acute stages of nitrogen deficiency they respond to the application of HNO_3 to the soil without showing leaf areas that fail to become green. Young budded Valencia orange trees were grown in Sierra loam in large galvanized iron containers with suitable drainage. After a year's growth the need of nitrogen was apparent. The soil of several cultures was given a solution of calcium nitrate in tap water whenever the trees showed the need of water. The concentration of calcium in the solution used was about 365 p.p.m. and that of nitrate was about 1,130 p.p.m. Whenever the concentration of the soil solution became excessive as shown by the wilting of the leaves, more of the calcium nitrate solution was added to dilute the more concentrated solution and allow some of the more concentrated solution to drain away. During the first two years of growth an occasional application of Hoagland's solution was made to replenish ions lost in the drainage.

The growth was of a healthy green color, no mottled leaves being evident at any time. Each day for the next two years, 5 gallons of tap water containing 5 cc of concentrated HNO_3 was given the soil in one of the containers. The residue of calcium from the use of calcium nitrate was sufficient to prevent the soil from becoming too acid, the pH of the drainage water at the end of the experiment being about 5. Figure 12 shows the luxuriant growth made by the Valencia orange tree that was given this excessive amount of nitrate. Although no humus was applied at any time, no mottling occurred and no difficulty was had in securing drainage. The excessive nitrate did not prevent the production of a large crop of fruit.

Calcium and Sodium Nitrate with Young Budded Citrus Trees.—In large tank experiments with Sierra loam, young budded Valencia orange trees were not injured by frequent large applications of calcium nitrate or of sodium nitrate. Figure 13 shows the excellent

type of growth of the trees in the nitrate-treated cultures at the end of six years; the leaves were in all cases free from mottling. No bad effects have shown themselves during the ten years of growth thus

Fig. 12. Valencia orange tree grown in a galvanized iron container filled with Sierra loam that received large amounts of calcium nitrate solution for two years, and daily for the next two years received 5 gallons of tap water containing 5 cc of concentrated HNO_3. Meter stick gives an idea of the size of the top. Note the dense foliage, absence of mottled leaves, and the size as compared with the tree of equal age shown in figure 14.

far, except in culture 8, where the pH of the drainage water began showing black alkali or alkalinity to phenolphthalein. Here there was a considerable loss of leaves with the subsequent production of small narrow leaves that were not mottled. In the controls there were

indications of mottle-leaf, which was partially masked by the yellowing that resulted from the nitrogen deficiency. The soil in these tanks, as in the smaller soil cultures, was kept moist as much of the time as possible, the excess water finding ready drainage. In the case of the sodium-nitrate-treated soil, drainage was excellent at all

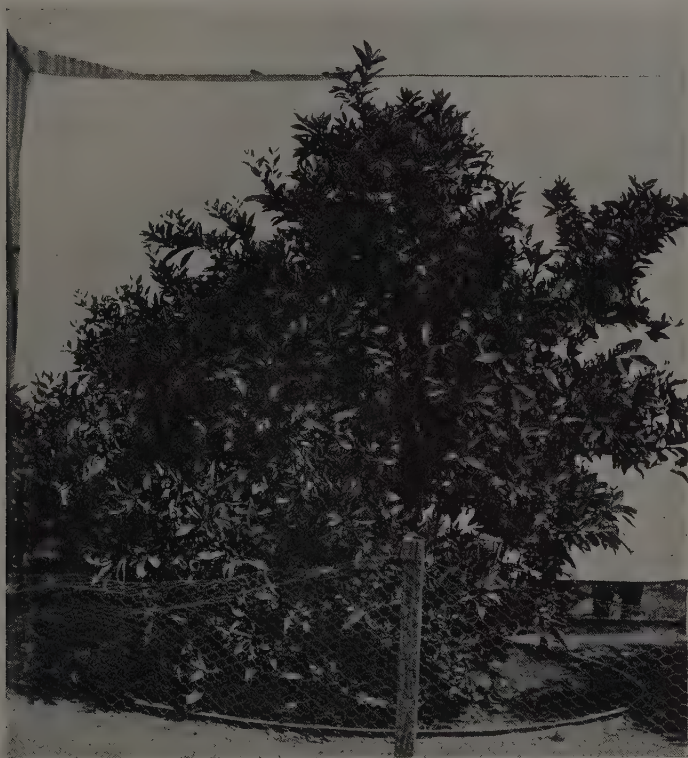

Fig. 13. Valencia orange tree six years in Sierra loam in a tank 8 feet in diameter and 4 feet deep, typical of the trees that received large amounts of either calcium or sodium nitrate under excellent drainage conditions (cf. figure 14).

times provided the soil was kept moist. When allowed to become somewhat dry, the soil would not take water readily; therefore the soil was kept moist almost continuously.

The Valencia orange trees were planted in these large tank cultures 8 feet in diameter and 4 feet deep on May 21, 1920, and are still under culture. Two cultures have received only tap water during

TABLE 1

COMPOSITION (EXPRESSED IN PARTS PER MILLION) AND pH VALUE OF DRAINAGE WATER FROM SOIL CULTURES IN LARGE TANKS

Soil treatment	Culture	Na	K	Ca	Mg	Cl	HCO_3	NO_3	SO_4	Total solids	pH
September, 1924											
Control	1	194	30	273	97	156	1,198	2	288	1,730	7.6
Control	2	96	24	206	67	85	952	3	84	1,090	7.6
Calcium nitrate	3	292	54	1,289	375	237	859	4,145	340	8,280	7.6
Calcium nitrate	4	405	49	2,492	709	454	530	8,915	763	16,100	7.6
Calcium nitrate	5	265	47	1,779	554	246	416	5,985	509	11,440	7.6
Sodium nitrate	6	356	69	2,577	732	1,214	833	6,700	1,421	15,583	7.6
Sodium nitrate	7	439	74	3,121	949	1,084	1,123	8,390	1,367	20,609	7.6
Sodium nitrate	8	370	53	2,144	705	980	1,030	6,250	1,396	13,169	7.6
November, 1926											
Control	1	375	46	1,380	385	....	....	5,357	140	9,097	7.6
Control	2	182	47	202	93	....	....	9	177	1,338	7.6
Calcium nitrate	3	526	29	183	86	....	....	538	230	2,353	8.2
Calcium nitrate	4	606	89	1,8,3	512	....	....	8,375	309	12,138	7.4
Calcium nitrate	5	204	45	1,649	416	....	....	6,954	82	10,288	7.8
Sodium nitrate	6	867	108	335	138	....	....	1,709	304	4,629	7.8
Sodium nitrate	7	147	23	179	76	....	....	79	136	1,105	7.4
Sodium nitrate	8	935	69	276	145	....	....	1,233	563	4,421	8.4
December, 1927											
Control	1	114	17	112	48	....	....	....	....	....	7.0
Control	2	134	16	102	45	....	....	....	....	....	7.8
Calcium nitrate	3	86	16	111	31	....	....	....	....	....	8.0
Calcium nitrate	4	282	22	481	149	....	....	....	....	....	7.4
Calcium nitrate	5	101	15	113	40	....	....	....	....	....	7.5
Sodium nitrate	6	414	43	95	44	....	....	....	....	....	7.3
Sodium nitrate	7	296	37	70	36	....	....	....	....	....	7.6
Sodium nitrate	8	367	31	72	35	....	....	....	....	....	8.3

the entire period, in which time only 2 fruits were produced. Whenever new growth began, its growth was made at the expense of older growth. Three cultures each received 1,181 grams $Ca(NO_3)_2 \cdot 4\ H_2O$, and 3 each 425 grams $NaNO_3$ at least twice each year: in March and September. These applications are on an equimolecular basis, with the calcium nitrate cultures receiving applications of 620 grams of NO_3 or 140 grams of N, and the sodium nitrate cultures half this amount. Drainage water was collected at intervals. The composition of that collected in September, 1924, in November, 1926, and in December, 1927, will serve to show the magnitude of the concentrations of the ions after four, six, and seven years respectively.

Table 1 indicates the difficulty of leaching large soil cultures equally, as is shown by the large variations in the concentration of total solids in the drainage water. The nitrate concentrations were excessive in the drainage of the nitrate-treated cultures, and in one control culture (1926) the concentration became excessive, as is also shown in the total solids. In 1924 the loss of calcium from the sodium-nitrate-treated cultures was as great as, or greater than, that of the calcium-nitrate-treated cultures.

In 1926 there was a falling-off in the calcium, except in the control cultures, and an increase in the sodium of the drainage water. Only in culture 8 did the pH exceed that of the turning point of phenolphthalein (8.3), which indicates the formation of a slight amount of black alkali.

In 1924 the drainage water from the sodium-nitrate-treated cultures showed huge losses of calcium from the soil, and the calcium was not replenished as in the case of the calcium-nitrate-treated cultures. The concentration of total solids in the drainage water at times reached a magnitude of 20,000 p.p.m. The ash of mature orange leaf sap that has been centrifuged free from suspended material far exceeds this concentration, as is seen from table 2. Calcium and potassium were found to be present in very large concentrations. Experience with soil containers has shown that roots penetrate into the drainage system, and appear to be in a healthy state. The drainage water may become concentrated gradually; this permits the withstanding of concentrated solutions that otherwise cause injury. When the concentration of solutions in which the roots of young walnut seedlings were placed was raised slowly over a period of time, it was found that the roots are able to withstand concentrations approximating the concentrations of these drainage waters.

TABLE 2

CONCENTRATION OF SAP CONSTITUENTS OF MATURE HEALTHY ORANGE LEAVES

Sample	Ash constituents as a per cent of ash					Parts per million of sap					
	K	Ca	Mg	SO_4	PO_4	Ash	K	Ca	Mg	SO_4	PO_4
1	14.49	24.98	2.39	5.15	2.09	53,650	7,773	13,400	1,283	2,763	1,123
2	14.93	25.40	2.41	5.19	2.61	53,012	7,916	13,464	1,280	2,748	1,384

TABLE 3

COMPOSITION OF VALENCIA ORANGE LEAVES COLLECTED OCTOBER 31, 1924

Culture treatment	Per cent of ash in dry matter	Per cent of total nitrogen in dry matter	Per cent of dry matter in fresh weight	Ash constituents as a per cent of total ash						
				Na	K	Ca	Mg	Cl	SO_4	PO_4
Control	15.58	1.65	36.78	3.43	10.37	27.33	2.45	0.48	4.35	11.69
Calcium nitrate	14.99	2.90	38.62	0.71	4 15	33.39	2.63	0 25	2.25	4.04
Sodium nitrate	14.60	2.56	42.86	0.74	5.27	31.40	2.54	0.33	2.28	4.03

The effect on leaf abscission, the formation of small green leaves on one of the sodium-nitrate-treated cultures, and the tendency to mottle on the control trees has already been noted. Leaves collected October 31, 1924, had the composition shown in table 3. The table shows the reduced calcium and high potassium and phosphate of the control leaves, which are typical of leaves that are mottled, as described by Kelley and Cummins.[22]

The large applications of calcium nitrate did not increase the calcium above the normal for healthy mature Valencia orange leaves. The total N of the leaves of the calcium-nitrate-treated cultures is higher than that for the sodium-nitrate-treated cultures that received half as much NO_3, but both are normal for healthy, vigorous young trees. The sodium applications had not at that date increased the concentration of sodium in the leaves.

In August, 1927, mature leaves were again collected from the calcium and sodium-nitrate-treated cultures 5 and 8 respectively, that were previously mentioned in table 1. The leaves from culture 8 were dark green but somewhat burned and the new leaves were small but not mottled. The results given in table 4 show that when the calcium is low in mature Valencia orange leaves of the sodium-nitrate-treated culture they are not mottled. All cases as yet found with orange leaves when the method of leaf sampling was adequate confirm the results of Kelley and Cummins[22] that mottled orange leaves have a reduced calcium content. Table 4, however, shows that mature orange leaves with a reduced calcium content are not necessarily mottled. The table further shows that the leaf burn was primarily due to an increased absorption of sodium and a decreased absorption of calcium. The large absorption of sodium may also have reduced the absorption of potassium. No mottled orange leaves have as yet been found to contain such a large concentration of sodium. Mottled leaves are always richer in potassium than are normal leaves.

Figure 14 illustrates the type of growth obtained when a solution of sodium nitrate in tap water is used on Sierra loam cultures in containers 20 inches in diameter and 24 inches deep, and no special care is taken to keep the soil moist on the surface or to provide enough solution to drain off and remove excess salts. The solution applied contained 824 p.p.m. Na and 2,214 p.p.m. NO_3. Under such conditions there is a gradual loss of leaves and the new leaves are very weakly mottled and are much undersized. Upon abscission the subsequent new leaves are not mottled at all, but are narrow and tipburned (cf. with control tree of equal age shown in figure 12). The composition

TABLE 4

COMPOSITION OF VALENCIA ORANGE LEAVES (NOT MOTTLED) COLLECTED AUGUST 6, 1927

Treatment	Culture	Leaf condition	Per cent of ash in dry matter	Per cent of dry matter in fresh weight	Ash constituents as a per cent of total ash			
					Na	K	Ca	Mg
Calcium nitrate	5	Healthy	17.46	42.11	1.59	5.54	34.37	2.00
Sodium nitrate	8	Tipburned	13.24	41.94	10.61	4.30	28.50	1.87

TABLE 5

COMPOSITION OF VALENCIA ORANGE LEAVES FROM SOIL CULTURES TREATED WITH NITRATES

Treatment	Number of cultures treated	Ash as a per cent of dry matter	Ash constituents as a per cent of total ash						
			Na	K	Ca	Mg	Cl	SO_4	PO_4
Calcium nitrate	6	14.18	0.69	4.74	32.89	1.26	0.19	1.42	2.26
Sodium nitrate	6	14.57	6.58	4.69	28.02	3.19	0.21	2.07	1.45
Calcium nitrate and sodium nitrate	6	15.05	5.49	6.12	28.91	2.70	0.36	2.19	1.47

of the normal leaves of these trees in soil cultures, six of which received sodium nitrate, six the molecular equivalent of sodium nitrate as calcium nitrate, and six equal quantities of both salts, is given in table 5. These results agree with those of leaves from Valencia orange

Fig. 14. Valencia orange tree in Sierra loam soil cultures that received nitrate of soda with insufficient drainage.

trees in large tank cultures reported in this paper. The percentages of calcium are reduced where sodium is used but the percentage of sodium is increased, the potassium percentage not being much affected. It should be recalled that in mottled leaves the reduction in the percentage of calcium is accompanied by an increase in the percentage of potassium.

When the soil of sodium-nitrate-treated soil cultures in such condition is calcium-nitrate-treated, the penetration of water is increased, but there is loss of nearly all of the leaves, and the production of fewer but small, narrow, tipburned leaves. After a year the growth was only slightly improved and was far from that obtained with calcium nitrate alone. When the calcium nitrate was applied after two years' use of sodium nitrate, the setting-free into the soil solution of excessive amounts of sodium at one time through base exchange brought about changes in alkalinity, solubility, etc., so that temporarily at least the conditions for growth were made worse.

As previously shown, whenever faint alkalinity to phenolphthalein developed, injury to the tree followed as a consequence. Valencia orange trees in Sierra loam soil cultures that at first were given a solution containing 365 p.p.m. Ca and 1,130 p.p.m. NO_3 and were as vigorous and free from mottle-leaf as the tree shown in figure 12, lost most of their mature leaves when powdered $CaCO_3$ and $Ca(OH)_2$ were added to the soil until faint alkalinity of the drainage water to phenolphthalein developed.

If sodium-nitrate-treated soils are given distilled water in amounts barely sufficient for drainage, their drainage water may turn pink to phenolphthalein, while if considerable leaching occurs the pH may fall considerably below this point and the injury becomes less or is absent. Several large tank cultures with Sierra loam such as that shown in figure 13, as well as smaller soil cultures, were given $CaCO_3$ in addition to the calcium nitrate, and the pH of the drainage water was alkaline to phenolphthalein. Likewise NaOH and Na_2CO_3 were given in addition to sodium nitrate.

In each case there was a much more abundant leaf abscission as compared with the control and calcium or sodium-nitrate-treated cultures, and although the drainage was excellent it was not an easy matter to bring about recovery because of root injury under the highly alkaline conditions.

Dried Blood and Sodium Nitrate on Citrus Seedlings in Soil from Rubidoux Plots.—Containers of about 110 pounds capacity were filled with soil taken from plot C (dried blood plot) and plot H (sodium nitrate plot) of the Rubidoux trials at the Citrus Experiment Station and planted with St. Michael orange seedlings on July 28. The cultures were grown until the following May 7 with the addition only of distilled water to maintain proper moisture conditions. No drainage water was allowed to escape and care was taken not to have any excess water. Figure 15 shows a comparison of the growth made on the two soils.

The plot H seedlings were much smaller than the plot C seedlings and lost many old leaves that burned. There was considerable new growth on the plot H seedlings but as the leaves became mature they burned and abscissed. The type of growth can hardly be a result of differences in nitrate content of the two soils, for when plot H soil was placed in containers, budded Valencia orange trees planted, and the cultures leached somewhat with distilled water to remove salts, excellent growth resulted with no leaf burning or premature abscission. It has been shown by Haas[8] that there is a lack of agreement

Fig. 15. St. Michael orange seedlings in cultures of soil from plots C and H of the Rubidoux trials at the Citrus Experiment Station. Plot C is treated with dried blood and plot H with nitrate of soda, the actual nitrogen applied per tree on both plots being 1.35 lbs. a year.

between the barley-producing power of soils from these plots and the yield and condition of citrus on such soils at the time the samples were taken. The amount of nitrogen previously added to the soil was not found to be an accurate measure of its barley-producing power. The trees in plot H are in much poorer condition than those in plot C. It is evident from figure 15 that citrus seedlings grown in soil taken from plots C and H show the same order of growth as the trees growing on the plots from which the soil was obtained.

Effect of Various Nitrogen Treatments on Covercrops of Rubidoux Plots.—For the past several years various covercrops have been grown as green manures on the Rubidoux plots of the Citrus Experiment Station. It seemed of interest to study the composition of the *Melilotus indica* plants grown on the various plots in order to learn whether the composition varies with the plot treatment. Vaile[42] has summarized the effect of the fertilizer treatments on the growth and yield of the citrus trees of these plots, although much information is

TABLE 6

COMPOSITION OF ASH OF MELILOTUS INDICA TOPS FROM RUBIDOUX PLOTS OF CITRUS EXPERIMENT STATION

Plot	Weight of tops, in grams		Treatment of soil over a period of years	Ash as a per cent of dry matter	Ash constituents as a per cent of total ash						
	Fresh	Dry			Na	K	Ca	Mg	Cl	SO_4	PO_4
L	379	63	Nitrate of soda, blood, and sulfate of potash	11.31	11.03	33.93	6.56	2.49	7.80	9.67	9.68
I	367	57	Muriate of potash*	12.15	10.03	32.52	8.10	2.72	7.41	11.44	8.94
Q	270	45	Nitrate of soda, blood, superphosphate, and sulfate of potash	11.50	9.72	32.48	7.65	2.40	6.98	9.05	12.72
M	405	66	Check	12.84	11.19	32.36	7.58	2.84	8.60	9.15	8.71
J	334	55	Superphosphate	11.33	9.75	32.34	8.19	2.67	5.99	9.00	12.31
D	364	62	Sulfate of potash	12.06	9.31	32.05	7.57	2.63	7.28	10.13	8.35
C	462	74	Dried blood	12.43	9.82	32.00	6.87	2.36	7.12	8.55	9.23
K	514	84	Steamed bone and sulfate of potash	12.92	9.28	30.79	7.35	2.66	7.62	8.47	8.08
G	449	76	Nitrate of soda, blood, and bone	11 44	9.56	30.70	8.45	2.76	7.65	9.50	9.45
H	374	59	Nitrate of soda	12.07	11.90	30.44	7.17	2.38	9.61	9.45	9.64
R	294	49	Sulfate of potash	11.66	8.94	29.84	9.34	2.76	8.36	9.60	7.88
N	530	89	Superphosphate and blood to equal nitrogen in bone plots	11.23	10.24	29.69	9.01	2.50	7.85	8.68	10.64
E	314	52	Steamed bone	11.79	12.70	29.40	7.47	3.22	6.98	9.90	9.96
B	430	71	Check	12.03	9.69	29 21	8.17	2.73	7.26	9.97	8.96
P	357	63	Steamed bone	10.54	10.26	27.35	8.30	2.69	9.19	10.52	9.58
S	335	56	Dried blood	10.14	10.85	27.02	9.59	3.83	8.25	10.44	9.26
T	374	65	Check	10.34	10.60	26.91	10.99	3.42	8.25	8.34	9.30
A	432	75	Nitrate of soda, blood, bone, and sulfate of potash	11.41	9.95	26.87	9.68	3.54	5.92	7.32	8.63

* Sulfate of potash 1920–1921.

still to be supplied regarding the effect of the soil variation, which in some cases is marked.

The tops of the *Melilotus indica* plants were obtained in the spring of 1925 just prior to the plowing under of the covercrop. Table 6 makes it clear that the ash constituents bear no consistent relation to fertilization of the plots. Total nitrogen determinations[3] likewise were remarkably constant. This plant thrives whether citrus trees do well or poorly on the same soil and is far less sensitive to soil conditions than citrus trees or seedlings. This may be due to a greater ability of the *Melilotus indica* plants to regulate the absorption of ions that are injurious at certain concentrations to citrus.

The high sodium and potassium values are of interest, as well as the relatively low percentages of magnesium and calcium, the latter values in each case being the higher. The results indicate that in addition to having about 3 per cent of total nitrogen in the dry matter, the tops contain large amounts of other elements which are drawn from varying depths to the top of the soil. In general the higher potassium values are associated with the lower calcium values as is the case in the ash of citrus leaves. These *Melilotus* tops were immature when plowed under, the succulent condition being desirable for green-manure purposes.

In several of the plots there was a considerable growth of *Chenopodium murale* (ragweed) among the *Melilotus indica* plants that were much more mature. The tops of these ragweed plants were collected at the same time as the others and were analyzed. The total nitrogen in the dry matter was approximately the same as that for *Melilotus indica.* Table 7 shows the composition of the ash of these plants. The plots have been arranged in such a manner as to make the data more comprehensible, the order being determined by the sodium values. It should be stated that dried blood is rich in sodium chloride and that steamed bone is sulfuric-acid treated. Plots O and P appear to be out of place as regards sodium and it is of interest that the potash plots are not found in the lower portion of the list of treatments. The ash in the dry matter and the potassium in the ash roughly decrease as the sodium increases, while the remainder of the columns show no obvious relation.

Even though both lots of plants were of the same age, the ash of ragweed was by far the richer of the two, partly because of the larger leaves and the more rapid maturing of the ragweed plants. In their

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[3] Total nitrogen determinations of *Chenopodium murale* (ragweed) and *Melilotus indica* plants were made by E. E. Thomas of the Division of Agricultural Chemistry.

TABLE 7

COMPOSITION OF ASH OF CHENOPODIUM MURALE TOPS FROM RUBIDOUX PLOTS OF CITRUS EXPERIMENT STATION

Plot	Weight of tops, in grams		Treatment of soil over a period of years	Ash as a per cent of dry matter	Ash constituents as a per cent of total ash						
	Fresh	Dry			Na	K	Ca	Mg	Cl	SO_4	PO_4
N	302	42	Superphosphate and blood to equal nitrogen in bone plots	24.02	12.33	32.39	5.12	5.67	5.94	3.55	7.94
F	530	96	Stable manure	19.34	12.63	31.38	4.89	6.56	9.08	3.32	5.57
D	310	47	Sulfate of potash	20.75	13.24	33.96	4.19	6.02	8.91	3.17	5.20
M	422	63	Check	21.56	13.50	34.39	4.00	6.22	8.47	3.43	5.34
K	362	61	Steamed bone and sulfate of potash	20.97	14.07	29.11	5.26	6.42	7.97	3.25	4.87
I	519	82	Muriate of potash*	20.40	15.35	28.53	5.15	6.03	10.81	3.29	5.43
E	400	60	Steamed bone	20.92	15.93	28.44	5.04	7.41	7.71	3.36	5.30
R	272	50	Sulfate of potash	17.44	16.55	25.92	6.06	6.22	12.11	4.32	5.71
L	479	90	Nitrate of soda, blood, and sulfate of potash	19.69	17.91	23.19	4.96	5.28	7.97	3.40	5.14
A	430	85	Nitrate of soda, blood, bone, and sulfate of potash	18.05	18.14	16.70	7.19	5.62	8.11	4.05	5.77
O	447	82	Stable manure and rock phosphate	18.16	18.81	23.37	4.44	6.86	10.52	3.69	5.40
P	290	52	Steamed bone	17.32	19.69	21.50	5.84	6.41	11.21	3.52	5.42
C	525	87	Dried blood	17.11	20.06	20.89	5.43	6.81	9.82	3.65	6.22
S	372	65	Dried blood	17.29	21.64	16.01	5.23	8.33	9.26	3.68	5.96
G	377	70	Nitrate of soda, blood, and bone	16.16	22.06	18.44	6.27	6.53	8.23	3.72	6.53
Q	530	89	Nitrate of soda, blood, superphosphate, and sulfate of potash	19.40	22.99	16.56	4.47	6.62	9.66	3.66	6.20
H	602	127	Nitrate of soda	16.34	23.45	14.46	6.50	6.69	7.30	3.95	6.03

* Sulfate of potash 1920–1921.

ash, the ragweed tops had higher percentages of sodium and magnesium but lower percentages of potassium, calcium, sulfate, and phosphate than *Melilotus indica*. Ragweed tops with the highest percentages of sodium and potassium were not necessarily those which were lowest in calcium, differing from *Melilotus indica* and citrus leaves in this regard.

The absorption of sodium by the ragweed plants, as reflected by the tops, parellels the condition found in citrus: Vaile[42] showed that the use of nitrogen in the form of sodium nitrate, or blood without the use of bulky organic manure of some sort was conducive to the development of mottle-leaf. Elsewhere in this paper it is shown that mottled citrus leaves may have a slightly increased absorption of sodium. In this regard the use of green manures, while conserving much of the nitrogen, potassium, and phosphorus that might be leached below the root zone during the autumn and winter by rains in conjunction with irrigation water, also conserve undesirable elements.

CITRUS LEAF CONDITION AS A RESULT OF LEACHING SOIL WITH INORGANIC ACID SOLUTIONS

Acids: Dilute Sulfuric or Phosphoric.—The beneficial effects of sufficient drainage to prevent alkalinity to phenolphthalein and thereby prevent injury to citrus have previously been considered. It has also been shown that when Sierra loam was treated with calcium nitrate solutions for several years and then leached daily for several years with 5 gallons of water containing 5 cc of concentrated HNO_3 excellent growth resulted. The pH in this case did not go below 5. The injurious effects upon citrus of a too prolonged leaching of the soil with dilute acids such as sulfuric and phosphoric will now be considered.

The effect of leaching Sierra loam in 12-gallon earthenware pot cultures (planted to budded Valencia orange trees) with 5 and 10 cc of H_2SO_4 or H_3PO_4 in 18 liters of tap water is shown in figure 16. The leaves became pale green and in some regions the chlorophyll was practically absent. When this condition was brought about at pH values below 4, large applications of the calcium nitrate solution of the strength used in the other soil cultures were made. All of the leaves became green except in areas where injury was most severe. Many of the leaves in becoming green passed through stages of mottling. None of the leaves ever became dark green, but after the

application of calcium nitrate solution many were covered with resinous areas as shown in figure 16. The acids no doubt gradually depleted the soils of many of the essential ions so that the application of calcium nitrate alone was inadequate to bring about rapid recovery of the older leaves, although the new leaves produced were normal.

Fig. 16. Valencia orange leaves from soil cultures leached with weak sulfuric or phosphoric acid. The addition of calcium nitrate solution increased the chlorophyll in tissues not severely injured and brought about the production of resin or gum in severely injured tissues.

The effect of excessive acidity in sand cultures with young budded Valencia orange, grapefruit, and lemon trees was a general yellowing of the leaves. This occurred even when the culture solution contained a nitrate content equal to that of the unmodified Hoagland's solution. In this case the excessive acidity resulted from the substitution of iron nitrate for various portions of the calcium nitrate used in Hoagland's solution. The sulfate ion of solution A of Hoagland's solution brought about an increased precipitation of the iron and the acidity of resulting culture solution soon was below pH 4, which is outside the range of pH best suited for health in citrus. Even though the original culture solution contained an abundance of soluble iron and

nitrate, the excessive degree of acidity together with the precipitating action of the sulfate ion, made the leaves yellow as though in need of iron or nitrate. The injurious action of salts of sulfuric acid is further referred to under the heading of calcium sulfate.

Dilute Culture Solution Saturated with CO_2 but Lacking Calcium. —When cultures of Sierra loam (planted to Florida sour orange seedlings) were leached with a solution equal to one-tenth the con-

Fig. 17. Florida sour orange seedlings after eighteen months in Sierra loam: left, soil leached with one-tenth strength Hoagland's solution modified so as to omit the calcium nitrate and saturated with CO_2; right, control given optimum amount of distilled water. Premature abscission is a characteristic symptom of mottled leaves.

centration of Hoagland's solution modified to omit the calcium nitrate and then saturated with CO_2, the seedlings after eighteen months had the appearance shown in figure 17, left. The abscission of the oldest leaves has taken place although the terminal growth has continued. The control to the right shows healthy growth. The abscission can hardly be attributed to the loss of NO_3, PO_4, or K, since these are added in the solution applied. In the absence of adequate boron, growth would not have taken place and certain symptoms would have become manifest. The carbonated calcium-free distilled water leached away the soil calcium. This process was favored by the excellent drainage that was brought about by the daily application of 3 liters of solution to each of three wash-boiler containers of soil. The pH of the drainage water of the containers was between 6.8 and 7.0. The reduction in the soluble calcium was perhaps largely responsible for the abscission.

CITRUS LEAF CONDITION AS A RESULT OF ORGANIC ACID TREATMENT OF SOIL

Gum formation in the leaves of budded Valencia orange trees in 10-gallon containers of Sierra loam was brought about by single applications of organic acids to the soil without any leaching. A single application of 5 grams of salicylic acid or of 10 grams of oxalic acid was sufficient to cause the production of a hard gum on

Fig. 18. Gum formation on the dorsal surface of Valencia orange leaves from trees in soil that received a single application of salicylic or oxalic acid.

the leaf surface. Figure 18 shows the location of the gum on the dorsal surface of the leaves. The leaves were not dark green in color and in some cases showed a lack of vigor and an injurious effect.

Organic acids at this concentration could scarcely be expected to produce injury as a result of excessive acidity because of the weak ionization of such organic acids. Plant cells, however, are very permeable to organic acids. It has been shown by Haas[7] that an organic acid such as salicylic when of the same total acidity as that of a strongly ionized inorganic acid such as HCl, actually penetrates plant cells more rapidly.

CITRUS LEAF CONDITION AS A RESULT OF THE ADDITION OF CERTAIN TOXIC ELEMENTS TO SOIL

When salts containing chromium as the reagent were applied to 12-gallon Sierra loam cultures of Valencia orange trees it was found that neither 1 nor 10 grams of chromium nitrate had any bad effect on the growth. The chromium either entered the exchange complex or was precipitated out as the hydroxide. In any case it did not injure the trees, probably because it did not reach the root system, for in water cultures, chromium as a positive ion is injurious in relatively low concentrations.

Either 1 or 10 grams of potassium chromate quickly brought about injury. The lower strength caused permanent wilting even though the soil contained optimum moisture. The wilted leaves were retained by the trees for several months before abscission began, which gradually ended in the death of the trees. At the higher concentration the death of the trees resulted within a few days.

The electric charge on an ion is of considerable importance, therefore, in determining whether the ion will be toxic or noninjurious. Thus far the writer has been able to produce mottle-leaf in orange leaves on trees in Sierra loam to which sodium aluminate was added. In this case the aluminum-bearing ion is negative and the solution is alkaline. Such treated soil became impervious and the pH of the 1:2 water suspension was between 8.5 and 9.0. The aluminum as the positive ion in strong acid and in weak acid salts is being tested.

The hypothesis that mottle-leaf of orange trees may result from the absorption of toxic elements receives some additional support from preliminary Sierra loam soil cultures. Those cultures that were treated with excessive nickel, arsenic, zinc, acid aluminum salts, calcium hydroxide, or sulfate, show varying degrees of mottle. Other elements show promise in this direction, but it would be premature to make a definite statement regarding these except to mention that in these cultures no drainage water was allowed to escape at any time. This technique imitates the field practice of irrigating only to the depth of the root system and therefore does not remove from the radius of root absorption the ions that do not favor health in the tree.

CHLOROSIS

Effect of Iron Spray.—When chlorotic leaves (chlorophyll faded out generally over the entire leaf area except possibly at the veins) are sprayed with nicotine sulfate spray preparation which is rich in soluble iron, wherever the droplets of spray become concentrated upon evaporation, green areas of chlorophyll become visible, scattered

A *B* *C*

Fig. 19. *A*, Chlorotic leaves (chlorophyll faded out generally over the entire leaf) showing green areas resulting from droplets of iron solution catalyzing the formation of chlorophyll after becoming concentrated and penetrating the leaf surface.

B, Valencia orange leaves from trees in sand cultures that received 20 p.p.m. Cu as $CuSO_4$ in the culture solution. The chlorophyll distribution resembles a mosaic effect.

C, Mosaic-like spotting of lemon leaves from trees in sand cultures deficient in boron.

over the entire leaf blade (fig. 19*A*). It is possible that the use of a spreader in the spray might increase chlorophyll production in larger areas, but the effect does not appear to carry over on to the new growth. Similar results may be obtained with the other iron solutions. It should be mentioned here that iron salts dissolved in glycerine have been painted on the bark of citrus trees in the hope of obtaining penetration of iron. Excessive concentrations of iron are accompanied by gum exudation. Opportunity has not been

afforded as yet to test the efficiency of this simple method of iron application in overcoming chlorosis.

Mosaic Chlorotic Effect Produced by Copper Excess or Boron Deficiency.—The effects of spraying chlorotic leaves with materials rich in iron have resemblances to, but are different from, the spotted appearance that results on the leaves when Valencia orange trees are grown in sand cultures that receive Hoagland's solution plus 20 p.p.m. of copper added as copper sulfate. In the former case the green areas are more or less circular, conforming to the condensing or evaporating drop of solution on the leaf surface; in the latter case they are irregular in shape, the chlorophyll is destroyed here and there by the toxic concentration of copper, and the leaves have a mosaic appearance (fig. 19*B*). A leaf spotting that externally may be mistaken for a true mosaic has been produced in lemon leaves on trees in sand cultures receiving Hoagland's solution with a deficiency of boron (fig. 19*C*).

Relation to Mottle-Leaf.—In mottle-leaf the lighter areas are of a yellow color identical with that found in true chlorosis, in which the whole leaf area becomes yellow. A small amount of mottle-leaf has been observed in sand and soil cultures with citrus where calcium carbonate has been added, but it is soon lost and the leaves become chlorotic. In such cultures chlorosis is the usual condition. It is more intense when the cultures receive full sunlight. When Sierra loam soil cultures were leached with acids the leaves became somewhat chlorotic; when calcium nitrate solution was added to the soil, mottle-leaf developed as the leaves became green, but the mottle-leaf later disappeared.

In the field mild cases of mottle-leaf have been produced on large chlorotic Valencia orange trees that had never shown mottle-leaf previously, by injecting iron sulfate or tartrate solutions of excessive concentration into the trunks. This caused the abscission of most of the leaves and the production of a new crop of leaves that were slightly mottled while young, but healthy green with increasing age. While usually distinct phenomena in citrus leaves, the symptoms of some mottled and chlorotic leaves frequently become indistinguishable without observing the tree or grove as a whole.

In experiments with five-year-old Valencia orange trees in large tanks of Sierra loam soil it was found that by removing all fruit when of the size of a small pea, an unusually heavy vegetative growth was induced. The new leaves were somewhat chlorotic, then became weakly mottled as the amount of chlorophyll increased, and finally became dark green when fully mature.

The leaves of lemon cuttings in water cultures in which iron has become deficient ordinarily become chlorotic, although some mottling has also been found.

In composition the chlorotic leaves resemble mottled leaves, being low in calcium and high in potassium, sodium, and magnesium, as shown in table 8. This is most significant, for it establishes a relation

TABLE 8

COMPOSITION OF MATURE HEALTHY AND CHLOROTIC VALENCIA ORANGE LEAVES

Leaves collected	Ash	Ca	Ca	Mg	Na	K
	Per cent in dry matter		Per cent of ash			
Healthy leaves						
Irvine, Calif. May 17, 1926	20.93	7.75	37.02	1.42	....	....
Irvine, Calif. May 17, 1926	21.60	7.99	37.00	1.39	....	....
Irvine, Calif. October 6, 1927	22.64	8.03	35.49	1.73	1.19	3.78
Irvine, Calif. January 13, 1928	23.14	8.41	36.33	1.39	0.92	2.81
Chlorotic leaves						
Fillmore, Calif. January 28, 1926	13.45	3.12	23.19	2.29	3.95	19.20
Irvine, Calif. May 17, 1926	13.11	3.48	26.51	2.62	....	....
Irvine, Calif. May 17, 1926	13.33	3.54	26.57	2.56	....	....
Irvine, Calif. January 13, 1928	17.51	5.24	29.93	2.20	2.37	9.05
Tustin, Calif.	13.53	3.71	27.39	....	3.04	12.49

between chlorosis and mottle-leaf. In every badly mottled citrus tree are to be found leaves representing all gradations of mottle-leaf that range into the realm of chlorosis. The two therefore may represent different degrees of intensity of the same phenomenon, namely, a lack of chlorophyll. This lack of chlorophyll challenges present theories of catalysis in chlorophyll formation, about which very little is known at present. Iron, calcium, manganese, copper, ammonium, etc., have all been considered as being associated with chlorophyll formation and destruction, but the reactions involved in each case are not very clear.

SOURCES OF CALCIUM FOR CITRUS CULTURES

Calcium Chloride.—When calcium chloride is added as a source of calcium to the culture solution instead of the calcium nitrate in Hoagland's solution, the chlorine is absorbed in such large amounts that the leaf tips of Valencia orange trees in sand culture are burned as shown in figure 20 and the leaves later absciss. The absorption of calcium is essential for the health of citrus, but the form of the calcium salt used is most important.

Fig. 20. Valencia orange leaves from trees in sand culture receiving calcium chloride instead of the calcium nitrate of Hoagland's culture solution.

Chlorine has been found in tipburned Valencia orange leaves in the field to equal 3.58 per cent of the dry matter even where the calcium in the leaf ash was 33.24 per cent and the sodium 0.52 per cent. In such cases unless sodium carbonate was used in the determination, a value of 2.55 per cent of the dry matter was obtained, indicating the magnitude of the loss of chlorine in the ignition of leaves high in chlorine. The chlorine in the ash was 13.31 per cent. Such leaves absciss on shaking the branches.

Calcium Nitrate.—It is well known that citrus trees require large amounts of nitrogen. Reed and Haas[34] have shown with citrus trees in sand culture that when potassium is omitted from the culture solution the leaves are richer in calcium. It was shown in table 3

that large applications of calcium nitrate to large tank cultures of citrus in soil did not increase the calcium above the normal for healthy mature Valencia orange leaves. To maintain a high percentage of calcium in growing citrus leaves (roughly 35 per cent in the ash of healthy mature leaves) is of the greatest importance for the health of the trees. Although innumerable cases have been found in which citrus leaves have had subnormal percentages of calcium in their ash, only the case above referred to has been found in which calcium was above normal.

In order to study this matter further six containers 20 inches in diameter and 24 inches deep were filled with sand and planted to budded Valencia orange trees. These cultures were given Hoagland's

TABLE 9

COMPOSITION OF ASH OF VALENCIA ORANGE TREES GROWN IN SAND CULTURES THAT RECEIVED A SOLUTION HIGH IN CALCIUM NITRATE

Part of tree analyzed	Ash as a per cent of dry matter	Ash constituents as a per cent of total ash						
		Na	K	Ca	Mg	Cl	SO_4	PO_4
Leaves, immature	10.20	4.08	17.31	23.95	1.92	0.19	2.42	12.51
Leaves, mature	20.46	1.50	5.99	33.62	0.87	0.20	1.43	2.94
Shoots	7.92	1.43	5.40	33.67	1.22	0.12	1.66	8.27
Trunks	3.76	1.88	6.11	30.02	1.99	0.09	0.96	7.09
Roots	3.45	2.44	5.52	28.85	1.39	0.08	0.54	8.23
Rootlets (silica-free basis)	9.00	2.27	11.26	26.39	2.44	0.75	2.56	4.92

solution modified so as to use one-fourth strength of A and C solutions and 4.17 times the strength of solution B, the pH value of the culture solution ranging from 6.5 to 6.8. This solution had the following composition in parts per million: Na, 1.6; K, 46.0; Ca, 663.0; Mg, 13.5; Cl, 2.5; NO_3, 2,106.0; SO_4, 54.0; PO_4, 26.0; or a total concentration of 2,912.6 p.p.m. Traces of elements such as boron and iron were also present. This concentration of salts was not injurious, the trees appearing to be healthy and most vigorous.

With such a culture solution after several years of growth one might expect calcium to be absorbed by the leaves in large excess. This proved not to be the case. Table 9 shows the calcium content of the leaf ash to be that typical of healthy mature citrus leaves. The large amount of nitrate in the culture solution, which forced the production of new growth, appears to have prevented the calcium from accumulating. The ash in the dry matter decreases from the leaves to the roots inclusive but increases again in the rootlets, yet

the calcium gradient persists throughout. The rootlets are seen to be high in potassium, as is the case with immature leaves.

It is seen that by direct absorption from solutions high in calcium nitrate the leaves of citrus do not absorb supernormal amounts of calcium. In studies now being conducted on increased calcium absorption, manganese is being studied, since Kelley[(21)] and later McGeorge[(27)] have found manganese to stimulate a greater absorption of calcium by certain plants. The absorption of calcium in the absence of phosphorus is also being studied.

Fig. 21. Valencia orange trees grown in sand cultures with calcium carbonate as the source of calcium. The tree to the left is typical of the trees receiving Hoagland's solution with potassium nitrate instead of calcium nitrate; the tree to the right is typical of those in which sodium nitrate was substituted for the calcium nitrate.

Calcium Carbonate.—A series of 12 Valencia orange trees were grown in sand cultures with their supply of calcium in the form of finely powdered calcium carbonate that was well mixed with the sand, about 1,500 grams being added to each container 20 inches in diameter and 24 inches deep. A modified Hoagland's solution was added to each culture; in 6 the potassium nitrate was substituted for calcium nitrate, and in the other 6 sodium nitrate was substituted for the calcium nitrate. A mixture of the mono and dipotassium phosphates was used (pH 6.8) instead of the acid phosphate in order to lower the solubility of the calcium carbonate. The calcium carbonate did not dissolve at a rapid enough rate for the health of the trees even when the pH of the solution applied was changed to 6.0.

The potassium and sodium were both absorbed in large amounts with the result shown in figure 21. The potassium absorption was so rapid that the leaves soon absorbed enough to show injury and

absciss. The sodium absorption was not so injurious under the same conditions. Although the leaves eventually became somewhat chlorotic and growth was interfered with, the injury from excessive sodium was not so great as where potassium was in excess. The drainage water was slightly alkaline to phenolphthalein, which accounts for the chlorosis.

Analysis of the leaves that fell from the trees, which were treated with a culture solution rich in potassium was as follows:

Ash as a per cent of dry matter	Ash constituents as a per cent of total ash					
	K	Ca	Mg	Cl	SO_4	PO_4
15.74	40.16	7.46	2.76	0.35	3.28	2.78

The abnormally high K content and the low Ca content are obvious when it is considered that the ash of healthy mature leaves of Valencia orange trees contains approximately 3 to 5 per cent K and about 35 per cent Ca, quite the converse of the ash of these falling leaves. Some of the leaves of these high potassium trees were similar in appearance to mottled leaves that have been produced through the use of solutions high in potassium.

The effect of calcium carbonate in soil on citrus is frequently seen in the field as chlorosis. The soil may be impregnated throughout with calcium carbonate or the calcareous subsoil may approach the soil surface to varying degrees. The amount and distribution of calcium carbonate in the soil will frequently determine the age at which the trees will become worse.

Frequently trees that have been fully green for many years may become chlorotic even though the original soil may be deep and free from calcareous subsoil or scattered deposits of calcium carbonate in the upper layers of soil. In such cases the use of calcium nitrate has brought about the condition. The nitrate was absorbed at a more rapid rate than the calcium, some of which was precipitated at the root surface as a result of the excretion of carbon dioxide by the root. Instances have been found where the youngest or absorbing roots were encased by a calcium carbonate deposit. Effervescence resulted when lemon juice was applied. On breaking open a clod of soil taken from various depths in pits that were dug in such chlorotic areas, it was possible to trace the roots by the delicate white calcareous thread. Subsequent use of acid nitrogenous fertilizers or acid-forming soil amendments usually was all that was necessary to correct this

condition. Mixtures of calcium salts such as sulfate and carbonate have recently been found encasing and injuring citrus roots in the field. It is conceivable that when the soil solution is excessively rich in calcium salts the drying out of the soil may increase the injury. Such calcium precipitation no doubt seriously interferes with the exchange of solutes.

In water cultures in the glasshouse, phosphate has caused precipitation of calcium at the surface of the solution when the pH of the solution approached pH 7. In this case rotting of the roots resulted within a few days as a consequence of effective exclusion of gaseous exchange through this precipitate. The addition of weak HNO_3 in distilled water easily remedied the situation. The precipitation of calcium phosphate about a root is just as conceivable as at the surface of the solution in the pan culture. A study of the absorption of ions through such precipitation membranes may prove to be of considerable value.

Calcium Sulfate.—It has been shown by Haas and Thomas[17] that mottle-leaf can be produced in lemon leaves on trees grown in sand cultures in which there was considerable calcium sulfate. Some of these cultures were grown in large galvanized iron containers 20 inches in diameter and 24 inches deep in which the sand contained 500 grams of finely divided c.p. calcium sulfate. The addition of Hoagland's solution modified so as to have an increased calcium nitrate content permitted better growth than the reduction of calcium in the added solution or its exclusion. On such better-growing trees the mottling was more conspicuous, and there was a reduction in leaf abscission.

These experiments have been confirmed by many subsequent ones. The characteristic mottling seen in figure 22*A* has been produced in lemon cultures in which no calcium sulfate powder was added to the sand but in which a saturated solution of calcium sulfate was used in making up Hoagland's solution. In such cases the first 100 cc of drainage water obtained from the containers when the cultures were leached with distilled water had pH values ranging from 6.5 to 7.6. These values are higher than those obtained where the calcium sulfate powder was mixed with the sand and the culture solution added was a calcium-free Hoagland's solution.

When barium nitrate was occasionally added to certain calcium sulfate cultures, a reduction in the degree of mottling of the lemon leaves followed.

A mixture of calcium sulfate and of tricalcium phosphate in other lemon cultures gave less mottling than where calcium sulfate alone was used in conjunction with calcium-free Hoagland's solution.

It is of interest that no such mottling has thus far been obtained with orange trees in sand cultures involving calcium sulfate. In such

A *B*

Fig. 22. *A*, Lemon leaves from trees in sand cultures. Upper row: mottle-leaf resulting when calcium sulfate is present in large amounts. Lower row: burning or spotting that results when tricalcium phosphate is the source of calcium.

B, Spotted or burned Valencia orange leaves from trees in sand cultures. Upper row: from cultures in which calcium (as the nitrate) was low and sodium or potassium was high. Lower row: from cultures in which tricalcium phosphate was the sole source of calcium.

experiments the leaves may turn a pale yellowish-green, indicating that the excessive absorption of sulfate has made the absorption of nitrate relatively too small. This was also found to be the case when large concentrations of magnesium sulfate were employed in sand cultures.

Calcium Phosphate.—Lemon and orange trees were grown in containers 20 inches in diameter and 24 inches deep containing sand, in each of which 500 grams of finely powdered c.p. tricalcium phosphate

was mixed and to which was added Hoagland's solution modified so as to contain no calcium or phosphate. The pH of the first 100 cc of drainage water when such containers were leached with distilled water was about 7.5.

The absorption of calcium in the absence of phosphorus is being studied. Recently Davis[3] has shown that the calcium in apple twigs, barks, and wood was increased when phosphorus was omitted from the cultures.

In this experiment, as in the calcium sulfate experiments in which calcium nitrate was not added, there was a considerable abscission of leaves. No mottling developed in either lemon or orange leaves but there were numerous small, brown spots or burned areas. These are shown in figure 22*A*.

Such spots are no doubt related to the low availability of the calcium. They are analogous (fig. 22*B*) to those produced on leaves of orange trees in sand cultures with Hoagland's solution modified as to greatly reduce the calcium and greatly increase the sodium or potassium.

No determinations have as yet been made of the phosphate absorbed. Citrus absorbs large amounts of calcium and it appears that when the phosphate is not leached away the calcium cannot be obtained from this relatively insoluble source at a rapid enough rate to meet the high calcium requirements of citrus.

LOW-CALCIUM SOLUTION CULTURES WITH LEMON CUTTINGS

With High Potassium.—Lemon cuttings were grown in Hoagland's solution in which the calcium ranged from 5 to 50 p.p.m. instead of 159 p.p.m., as in the unmodified Hoagland's solution, and to which 312 p.p.m. K was added as KNO_3. After the cuttings had grown for two years the oldest leaves from all of the cultures were combined into one composite sample. These leaves had 10.18 per cent of soluble ash in the dry matter and 3.07 per cent of insoluble ash. This is approximately the converse of normal lemon leaves from the field, the values of which for the soluble ash are close to 5 per cent (for leaves of any age); for the insoluble ash they increase according to the age of the leaves (Haas[9]). The soluble ash, instead of being about 30 per cent of the total ash, was about 77 per cent.

The soluble calcium in the dry matter was only 0.13 per cent and the insoluble 0.61 per cent, in contrast to 1 and 5 per cent respectively

for healthy leaves. These percentages indicate that when calcium was deficient the ratio of the soluble to the insoluble calcium in the dry matter was approximately that found in the healthy leaves.

The insoluble calcium was 15.63 per cent of the insoluble ash when it should be 35 to 40 per cent. The total postassium was 5.43 per cent of the dry matter instead of 0.5 to 1.0 per cent as in healthy leaves, or 40.96 per cent in the ash instead of 5 to 10 per cent. Abscission of such leaves is premature.

The experiment emphasizes the tremendous effect that a deficiency of calcium may have on the absorption process. This is especially true in the cases of mottle-leaf and chlorosis. Most of the potassium in citrus leaves is water-soluble. This explains the extreme increase in the soluble ash of the dry matter. The insoluble ash consists largely of calcium, hence the insoluble ash is low when low calcium is used in the culture solution.

With High Magnesium.—When similar cultures were employed, using Hoagland's solution with calcium reduced to a range of 5 to 50 p.p.m. instead of the usual 159 p.p.m. of the unmodified Hoagland's solution, and 525 p.p.m. Mg added as $Mg(NO_3)_2 \cdot 6\ H_2O$, the soluble ash was 6.54 and the insoluble ash 4.04 per cent in a composite sample of the oldest leaves. The magnesium and potassium in the ash were 16.52 and 19.72 per cent respectively as contrasted with 2 to 4 per cent for magnesium and about 5 per cent for potassium in healthy leaves from the field. The leaves showed no burning but were somewhat chlorotic, while the roots were increasingly injured as the calcium concentration of the culture solution decreased.

HIGH-CALCIUM, LOW-POTASSIUM SOLUTION CULTURES WITH LEMON CUTTINGS

In water cultures with large lemon cuttings Hoagland's solution was modified as follows: double strength calcium nitrate was used, potassium nitrate was omitted, and the concentration of potassium was reduced to as low as 10 p.p.m. by the substitution of monocalcium phosphate (monobasic) for monopotassium phosphate (monobasic). It was found that the root growth was stunted and the laterals short and stubby, although not gelatinous. The pH of the solution was about 4.4, which is close to the lower limit of growth for citrus. Upon transfer to the usual culture solution, the cultures failed to make new growth. In some cases pruning away the old stunted root system brought about recovery of normal growth.

HIGH-POTASSIUM SAND CULTURES

With Normal Calcium of Hoagland's Solution.—When potassium nitrate is added to Hoagland's solution so as to give a total of 600 to 650 p.p.m. K and this culture solution is applied to young budded Valencia orange trees in sand, the leaves show an etching or dorsal-surface burning as shown in figure 23. High potassium has the effect

Fig. 23. Dorsal surface of Valencia orange leaves etched or burned from too high a potassium concentration in the solution applied to sand cultures.

of making the normal calcium concentration appear far too low for healthy growth.

The use of Hoagland's solution to which 781 p.p.m. K was added as KNO_3 on sand cultures of young seedling orange trees brought about mottling. Seedlings can withstand concentrations of salts that cuttings or budded trees cannot, and hence when subjected to high concentrations of a given element such as K, they are not so severely injured and continue to make some growth.

Mottled leaves have been produced through the use of solutions high in potassium. When Valencia orange cuttings were grown in Hoagland's solution with 312 p.p.m. K added as KNO_3, the leaves were normal and the roots were poor, but when Hoagland's solution was substituted without the additional KNO_3, the new leaves mottled

and the subsequent growth was normal. This emphasizes the fact that in slow-growing plants the establishment of equilibrium in the growth response to a given treatment may require considerable time. The

Fig. 24. Valencia orange tree grown in sand culture showing injury from excessive potassium. The leaves curled and became desiccated but remained attached for some time.

effect of the two culture solutions overlapped. This may be explained as follows: Growth was brought to a standstill because of the root injury resulting from the absorption of excessive K when the solution

contained the additional KNO_3. When the additional K was omitted, growth was resumed and the excess K absorbed by the injured roots could then pass out into the new growth and contribute to the production of mottle-leaf. When the K of the absorbing tissues was reduced sufficiently by the growth and equilibrium again was set up between the salts in the protoplasm and those in the new culture solution, the subsequent growth was healthy.

With Low Calcium.—Some young budded Valencia orange trees in sand cultures were given the following culture solution (parts per million): K, 1,004; Mg, 14; Na, 2; Ca, 15 (later raised to 60 with very little improvement); NO_3, 1,227; Cl, 3; SO_4, 54; and PO_4, 630; or a total of 2,949 p.p.m., to which was added 0.1 p.p.m. of iron and boron. The total concentration was approximately double that of Hoagland's solution and in itself was not found to be injurious; for young budded orange trees grow well in sand cultures with double strength Hoagland's solution. However, in the present cultures, the K and PO_4 much exceed double the concentrations found in Hoagland's solution, and the concentration of Ca is greatly reduced. Figure 24 shows the rolling and desiccation of entire leaves after only a few months' application of such a solution.

These results are of interest also in contrast with those of Reed and Haas[34] for sand cultures from which potassium was omitted. In their experiments the initial stages of injury were characterized by bronze-colored stripes on either side of the midrib, and later there were dead spots or scorch showing in the bronze-colored areas and a general destruction of chlorophyll. They also found that leaves of citrus grown in sand culture without potassium were rich in calcium and conversely leaves of trees to which no calcium salts were supplied were very rich in potassium. Wallace[43] has shown that leaf scorch of fruit trees is due to a potassium deficiency and is accentuated by nitrogen fertilization or fruiting. Reed and Haas[34] showed that Valencia orange leaves were scorched when there was present a deficiency of potassium in relation to calcium, and the present experiments indicate that the leaves are also scorched when there is an excess of potassium in relation to calcium.

HIGH-SODIUM, LOW-CALCIUM CULTURES

Sand Cultures with Citrus Trees.—The analysis of citrus leaves taken from trees growing in soil made saline with sodium chloride shows relatively small absorption of sodium as compared with the absorption of other bases such as calcium because there is usually a high concentration of soluble calcium present. The absorption of

Fig. 25. *A*, Valencia orange leaves from trees in sand cultures that received a culture solution containing a large concentration of sodium nitrate and a low concentration of calcium nitrate.

B, Lemon leaves from cuttings in water cultures containing large concentrations of sodium nitrate and low concentrations of calcium.

sodium is regulated largely by the concentration of calcium present, as is seen by the large amounts of sodium that accumulated in Valencia orange leaves taken from trees in sand cultures that received a culture solution high in sodium nitrate and low in calcium nitrate. The continued absorption of sodium brought about leaf burn, as shown in figure 25*A*.

The culture solution used was one-fourth the concentration of stock solutions A and C of Hoagland's solution, one-tenth the strength of stock solution B, plus 717 p.p.m. of Na and 1,930 p.p.m. NO_3. The composition of the culture solution in parts per million was: Na, 719; K, 47;

Ca, 15; Mg, 14; Cl, 3; NO_3, 2,032; SO_4, 54; PO_4, 26. The culture solution had a pH of 6.6, and the total concentration of 2,900 p.p.m. as such was not unfavorable to citrus in sand cultures; for young citrus trees make healthy growth in sand cultures receiving double strength Hoagland's solution. Considerable sodium was absorbed with 15 instead of 159 p.p.m. of calcium present in the culture solution. After one year's growth in the cultures the old leaves burned.

In the autumn of the second year of growth the trees were prepared for analysis. Table 10 shows the large absorption of sodium even by young immature leaves. The ash, sodium, potassium, chlorine, and phosphate are at a minimum in the trunk and increase in both

TABLE 10

COMPOSITION OF VARIOUS PORTIONS OF VALENCIA ORANGE TREES GROWN IN SAND CULTURES RECEIVING CULTURE SOLUTION CONTAINING 15 P.P.M. CALCIUM AND 717 P.P.M. SODIUM

Part of tree analyzed	Ash as a per cent of dry matter	Ash constituents as a per cent of total ash						
		Na	K	Ca	Mg	Cl	SO_4	PO_4
Leaves, mature and burned	14.50	24.95	14.50	14.32	2.00	0.29	0.79	7.31
Leaves, mature and not burned	13.96	18.29	16.43	14.73	1.61	0.29	1.24	5.50
Leaves, immature	9.73	17.42	23.86	8.60	2.12	0.25	1.40	11.36
Shoots	6.35	6.47	10.42	24.78	2.18	0.09	2.92	8.67
Trunks	3.33	5.72	5.94	23.26	1.97	0.09	2.62	5.70
Roots	3.61	11.89	6.08	22.40	1.61	0.11	1.23	7.10
Rootlets (silica-free basis)	7.84	9.95	11.93	20.26	3.48	0.75	3.63	9.36

directions. The calcium values increase from the rootlets to the shoots, but decrease in the leaves. The percentage of sodium in the ash of the leaves increases with the age of the leaves but that of potassium decreases.

Solution Cultures with Lemon Cuttings.—Lemon cuttings were grown in a culture solution, the composition of which in parts per million was approximately as follows: K, 217; Na, 534; Mg, 54; Cl, 10; SO_4, 216; NO_3, 1,644; and PO_4, 105; and in addition traces of iron as ferric tartrate and concentrations of Ca (as calcium nitrate) ranging from 5 to 55 p.p.m. were also present.

The leaves burned along the margin when the culture solution contained large concentrations of sodium nitrate and low concentrations of calcium nitrate. Figure 25*B* shows the effect of the gradual accumulation of sodium in the leaves by the progressive burning of new marginal areas as the accumulation becomes sufficiently great. The roots were in excellent health even though the leaves were badly burned in all of the concentrations of calcium.

TABLE 11

SODIUM IN LEAF ASH OF DIFFERENT GROUPS OF MATURE HEALTHY AND MOTTLED CITRUS

Variety	Condition of leaves	Sodium as a per cent of total ash															
		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12	Sample 13	Sample 14	Sample 15	Average sample
Lemon	Healthy	2.94	1.46	1 00	0 88	1.14	1.13	1 69	2.94	2.48	2.66	2.90	2.05	2.08	0.52	0.65	1.77
Lemon	Mottled	4.13	3.72	3.71	3.49	3.30	....	....	....	....	....	....	....	....	....	....	3.66
Navel orange	Healthy	2.48	2.38	2.47	1.39	0.80	1.61	....	....	....	....	....	....	....	....	....	1.86
Navel orange	Mottled	4.33	4.09	....	....	....	....	....	....	....	....	....	....	....	....	....	4.21
Valencia orange	Healthy	0.79	1.20	1.77	1.93	0.92	1.19	2.01	1.88	2.01	0.24	1.38	....	....	....	....	1.39
Valencia orange	Mottled	3.69	3.53	3.80	4.77	3.26	....	....	....	....	....	....	....	....	....	....	3.81

TABLE 12

COMPOSITION OF 30 CC SAP OF MATURE HEALTHY AND MOTTLED VALENCIA ORANGE LEAVES COLLECTED AT RUBIDOUX PLOTS, CITRUS EXPERIMENT STATION, JANUARY 17, 1928

Plot	Condition of leaves used	Ash, grams	Grams in dry matter				Ash constituents as a per cent of ash			
			Ca	Mg	Na	K	Ca	Mg	Na	K
C	Healthy	1.6005	0.4861	0.0470	0.0399	0.1332	30.37	2.94	2.50	8.31
C	Mottled	1.6092	0.4359	0.0371	0.0542	0.2047	27.08	2.31	3.37	12.72
G	Healthy	1.4988	0.4382	0.0416	0.0424	0.1430	29.24	2.78	2.83	9.54
G	Mottled	1.3464	0.3400	0.0350	0.0545	0.1999	25.25	2.60	4.05	14.84

Analysis of a composite sample of leaves collected from all of the cultures was as follows: insoluble ash 5.19, and soluble ash 8.11 per cent of the dry matter, quite the converse of the percentages found in the dry matter of healthy leaves in the field. Total calcium was 14.88 per cent of the total ash and insoluble calcium 22.74 per cent of the insoluble ash as contrasted with 35 per cent and 35 to 40 per cent respectively in the ash of healthy leaves in the field. The sodium was 18.70 per cent of the ash and the potassium 30.34 per cent. Even though the sodium in the culture solution was nearly double the potassium, the potassium found in the leaves was nearly double the sodium.

SODIUM AND MAGNESIUM IN RELATION TO POTASSIUM AND CALCIUM IN HEALTHY AND MOTTLED CITRUS LEAVES

Sodium was determined in the ash of healthy and mottled citrus leaves by Kelley and Cummins,[22] who considered their own results as not differing materially from one another. Examination of their data, however, shows indications of a larger sodium content in the ash of mature mottled Washington Navel and Valencia orange, lemon, and grapefruit leaves than in the ash of mature healthy leaves of the same variety. They found an average of 0.78 per cent in the ash of healthy Navel orange leaves and 0.60 per cent in the ash of healthy Valencia orange leaves. In the ash of the mottled leaves, the average percentages were 1.33 and 0.75 respectively. In the ash of healthy lemon and grapefruit leaves the average percentages were 0.42 and 0.25 respectively; in the ash of the mottled leaves the percentages were 0.88 and 0.29 respectively. Further support is given this hypothesis by the results given in table 11. The differences are not large but are consistently higher in the ash of the mottled leaves. It is realized that the method of obtaining sodium indirectly is subject to errors, but all of the analyses were made by the same analyst. The interest lies in the association of higher percentages of sodium with higher percentages of potassium in mottled leaves.

The analysis of sap of mature healthy and mottled Valencia orange leaves collected from the same trees on the Rubidoux plots of the Citrus Experiment Station may be of interest in this connection. The trees were located on plot C, which receives dried blood, and on plot G, which receives sodium nitrate, blood, and bone. Dried blood was found to contain 0.84 per cent sodium in the dry matter (dried at 80° C) and 0.56 per cent potassium. The ash soluble in nitric acid

was found to contain 18.73 per cent sodium and 18.35 per cent chlorine when sodium carbonate was used in the ashing for the latter element. Both plots receive sodium, and many of the leaves of the trees on each of the plots became badly mottled. Vaile[42] found in 1920 that 80 per cent of the leaves on the trees in plot C and 95 per cent of those on the trees in plot G were mottled.

Analyses of the sap from the mature healthy and mottled leaves are given in table 12. The results show lower percentages of the two divalent cations, calcium and magnesium, in the dry matter and ash of the sap of mottled than in those of healthy leaves, but higher percentages of the two monovalent cations, sodium and potassium. It is not possible as yet to conclude whether these results for magnesium will hold when more samples are tested. These results are contrary to those found by Kelley and Cummins.[22] Many analyses of magnesium in the ash of healthy and mottled citrus leaves have since been made by the writer but the results are not conclusive because the sampling of the healthy and mottled leaves was not done in a comparable manner. The results obtained by Haas and Halma[14] indicate the variation that occurs in the magnesium content of the ash of citrus leaves as the leaves increase in age and also demonstrate the necessity of comparable samples in work of this type. Furthermore, the separation of magnesium in the presence of large amounts of calcium is not an easy matter, even when the double precipitation of the calcium is made.

CALCIUM IN HEALTHY AND MOTTLED CITRUS

A study of the calcium content of mature healthy and mottled citrus leaves should prove of interest in that mottled leaves are always deficient in calcium. Data obtained by Haas and Halma[14] for healthy lemon and Washington Navel orange leaves when compared with those obtained by the writer for mottled leaves, make clearer the rôle of calcium in citrus leaves. The total ash and soluble ash of the healthy lemon leaves (table 13) vary with the increasing age of the leaves as shown by Haas and Halma.[14] The soluble[4] calcium calculated as a percentage of soluble ash is variable, but as the leaves become older the values become roughly constant at about 30 per cent, while the soluble calcium values for the mottled leaves are much lower, or approximately 11 per cent. If the insoluble calcium of the healthy lemon leaves is calculated as a percentage of the insoluble ash the values are approximately those obtained for mottled leaves.

[4] Water-soluble is meant unless otherwise stated.

The same situation holds in the case of Washington Navel orange leaves, as may be seen in table 14, except that the soluble calcium of the mottled leaves as a percentage of the soluble ash is much higher than in the case of lemon leaves (table 13). It is of interest to note

TABLE 13

RELATION OF CALCIUM TO THE ASH OF MATURE EUREKA LEMON LEAVES

Date	Ash as a per cent of dry matter		Soluble calcium as a per cent of soluble ash	Insoluble calcium as a per cent of insoluble ash
	Total ash	Soluble ash		
Healthy leaves				
1928—				
August 21	16.95	6.40	24.98	38.96
September 13	15.30	5.83	18.52	44.77
October 1	15.97	5.96	20.40	37.76
October 18	16.53	5.75	25.63	38.40
November 6	15.12	4.95	23.50	45.92
November 19	15.64	5.37	25.23	37.88
December 17	15.93	5.55	29.26	37.19
1929—				
January 8	15.89	5.64	25.01	37.27
February 11	15.45	5.32	32.63	38.36
February 26	13.52	4.62	25.19	46.96
March 25	15.62	6.29	29.24	37.41
April 29	16.39	6.75	30.30	46.26
May 29	20.98	7.76	29.37	39.03
July 8	23.58	7.01	29.69	39.41
July 23	24.18	8.43	30.57	39.37
August 12	24.77	8.26	32.03	40.22
August 27	25.14	8.18	29.97	39.15
November 5	25.56	7.65	29.51	39.59
Mottled leaves				
1927—				
September 6	14.24	6.14	10.07	37.41
September 6	13.78	5.41	12.41	37.51
September 6	14.29	5.73	13.08	37.15
September 6	14.47	5.57	11.32	39.10

the close agreement of the percentages of soluble calcium in the soluble ash of lemon leaves with those in the soluble ash of Navel orange leaves, especially as the lemon leaves advance in age. Perhaps the maintenance (within a given range) of a given percentage of soluble calcium among the soluble inorganic constituents in the cells of citrus leaves is a vital factor for the health of the leaves.

The insoluble calcium as a percentage of the insoluble ash remains approximately the same regardless of the variety or whether the

leaves are healthy or mottled. These results are of interest because the differences between the composition of healthy and mottled citrus leaves show a close similarity to those of healthy and mottled or rosetted walnut and pecan leaves as shown by Haas, Batchelor, and

TABLE 14

RELATION OF CALCIUM TO THE ASH OF MATURE WASHINGTON NAVEL ORANGE LEAVES

Date	Ash as a per cent of dry matter		Soluble calcium as a per cent of soluble ash	Insoluble calcium as a per cent of insoluble ash
	Total ash	Soluble ash		
Healthy leaves				
1928—				
August 21	19.23	10.57	25.11	37.41
September 13	13.03	7.05	30.05	40.97
October 1	17.55	9.85	28.64	38.31
October 18	15.68	9.10	28.39	34.48
November 6	15.99	9.68	30.45	34.65
November 19	17.35	9.40	28.74	38.74
December 17	16.28	9.24	29.37	36.65
1929—				
January 8	15.91	8.93	29.10	39.54
February 11	13.80	7.79	28.51	55.74
February 26	15.02	8.40	30.44	50.00
March 25	13.84	8.23	31.80	41.53
April 29	13.67	8.02	32.06	40.88
May 29	16.43	9.60	33.15	37.19
July 8	16.33	9.81	31.95	38.65
July 23	17.81	10.07	32.63	38.37
August 12	18.01	10.25	32.37	35.82
August 27	18.32	10.82	33.20	36.53
November 5	19.29	11.44	31.63	39.11
Mottled leaves				
1927—				
September 6	13.18	8.23	17.79	36.56
September 6	13.08	7.71	21.03	35.94
September 6	14.46	8.65	16.69	37.01
September 6	13.87	8.19	20.81	35.91
Plot H (Valencia orange)	16.24	10.98	21.62	36.04

Thomas.[13] Rand[33] found internal abnormalities of structure in extremely rosetted pecan leaves in which there was no differentiation into palisade and sponge tissue in the center of yellow spots, the tissue consisting of closely packed cells without air spaces. The writer was able to find such structures in mottled citrus, pecan, and walnut leaves but attaches little physiological significance to such structures for the following reasons: (1) These same structures were found occasionally in healthy leaves. (2) Cuttings having mottled citrus

leaves were rooted and during the rooting process the mottled leaves in most cases became dark green and healthy in appearance, although the shape of the severely mottled leaves did not undergo change. This increase of chlorophyll may be attributed to a reduction in light intensity when the cuttings were placed in rooting chambers, but the same improvement in color of the mottled leaves took place when the cuttings received very little reduction in light intensity. It is well known that mottled leaves in the field may become fully green later in their growth process. This is especially true in cases in which only the late autumn cycle of growth is mottled. It can be concluded therefore that abnormalities of structure of mottled leaves do not prevent such mottled leaves from becoming healthy leaves and from this it follows that either the abnormal structures become normal, which is unlikely in mature tissues, or that other tissues compensate for the abnormal structures by the production of a greater content of chlorophyll than is usual. The approximate constancy of the results for the insoluble calcium of citrus makes it unlikely that chemical cell-wall differences exist, although it does not preclude variations in the number of cells.

When citrus leaves are mottled, it is the soluble calcium that suffers reduction. The insoluble calcium requirement of the cell walls and other bodies or surfaces within the cells apparently is first satisfied before the liquid phase within the cell receives its full share of calcium. In this connection the results of Halma and Haas[18] are of interest. They found that the soluble calcium as a percentage of the soluble ash of rooted lemon leaves when grown in culture solutions containing different concentrations of calcium did not change to any great extent, but that the soluble potassium content increased enormously.

The relation of calcium to other elements in the trunk bark of healthy and mottled trees is being studied and the results will be presented in connection with other data in a later publication.

The leaves and fine rootlets represent both extremities of the tree and a study of the rootlets is important from the standpoint of the nature and amount of the elements that may eventually pass to the leaves. The material studied consisted of the smallest rootlets obtainable in the field at from 6 to 12 inches depth of soil. Two methods of cleaning the roots were used: (1) drying and then sieving away the soil after thorough rubbing; (2) repeated washing with tap and then with distilled water. The ash constituents are calculated as percentages of HNO_3–soluble ash, because of the large amounts of residual material.

TABLE 15

COMPOSITION OF NITRIC-ACID-SOLUBLE ASH IN YOUNGEST ROOTS OF LARGE CITRUS TREES IN THE FIELD, JULY 17, 1926

Soil treatment	Location	Kind of tree	Ash constituents soluble in HNO_3, as a per cent of total ash							
			Roots dry cleaned				Roots washed clean			
			Ca	Mg	Na	K	Ca	Mg	Na	K
Mottled leaves										
Sodium nitrate	Plot H, Rubidoux	Orange	15.56	3.77	4.41	15.77	14.91	4.38	10.09	10.62
Sodium nitrate	Plot H, Rubidoux	Lemon	21.63	3.10	3.34	9.74	22.06	3.88	8.48	7.94
Healthy leaves										
NaCl-rich irrigation water	Riverside	Orange	17.86	4.10	7.34	12.06	12.36	4.02	6.46	13.15
Check	Plot B, Rubidoux	Orange	.....	.....	.....	.....	13.02	5.10	7.74	16.35
Garbage	Fontana	Orange	20 85	3.84	2 20	11.82	29.53	4 39	0.88	3.83
Sodium nitrate	Fontana	Orange	23.45	2.40	7.39	6.79	25.58	2.51	4.82	6.05

TABLE 16

COMPOSITION OF WOOD AND BARK OF STOCK OF HEALTHY AND MOTTLED VALENCIA ORANGE TREES

Material	Part of tree from which material was obtained	Source of stocks	Condition of trees	Ash as a per cent of dry matter	Ash constituents as a per cent of ash			
					Ca	Mg	Na	K
Wood	Stock roots	Nursery	Heatlhy	2.53	11.39	3.28	2.19	13.73
Wood	Stock roots	Nursery	Mottled	1 52	21.39	4.69	4.93	12.46
Wood	Stock	Sodium nitrate and boron-treated soil cultures	Mottled	2.22	22.26	1.40	12.33	6.50
Bark	Stock roots	Nursery	Healthy	7.38	27.20	1.07	1.63	9.66
Bark	Stock roots	Nursery	Mottled	6.66	29.11	2.01	0.90	6.88
Bark	Stock	Sodium nitrate and boron-treated soil cultures	Mottled	8.63	31.96	1.09	3.70	2.03

Table 15 gives the values obtained for the analyses of the smallest roots. The calcium values are not as high, but the potassium frequently is much higher than in the leaves. The Fontana sodium-nitrate-fed roots were richer in sodium than the Fontana garbage-fed roots. The complications that enter into obtaining accurate young root samples free from adhering matter or loss of elements are so great that nothing can be learned from this source save perhaps an idea of the magnitude of the percentages involved.

A better way to study root composition was found to be the utilization of the sour orange stock of two-year-old budded healthy and mottled Valencia orange trees grown in the same nursery at Riverside, California. Each sample consisted of material taken from 10 trees. Only the tap root and lateral roots of equal size were used, all small roots being discarded. Only that portion of the tap root was used that was below the first secondary root, the aerial portion of the stock being discarded. It was possible with a fine brass wire brush to thoroughly clean the bark. The bark was then separated from the wood of the stock and both bark and wood samples were analyzed.

Healthy trees from the same nursery were grown in containers (20 inches in diameter and 24 inches deep) filled with Sierra loam that received sodium nitrate and boron so as to bring about severe mottling of the leaves with increasing leaf symptoms of boron injury (Haas[11]). Bark and wood samples of the ten sour orange stocks were taken in the manner just described and were also analyzed.

The boron was assumed to act as a poison in the cells of the leafy twig tissues, where it depressed the absorption of calcium and increased the absorption of potassium. This effect is typical not only of mature leaves and twigs of mottled citrus trees, but of young healthy leaves as well.

The data for the wood and bark samples given in table 16 indicate that the percentage of calcium is higher and that of potassium lower in the ash of the wood and bark of the roots of mottled trees than in healthy trees. This is the reverse of the conditions found in the ash of mottled and of healthy leaves. The calcium in the ash of the roots of mottled citrus trees may be higher because the leaves are unable to use all the calcium that has been absorbed by the roots, as a consequence of the accumulation of a toxic substance within the leaf cells. A known toxic agent such as boron has been used in soil cultures in the one case, with results similar to those obtained with mottled trees direct from the nursery. The higher potassium values for mottled than for healthy leaves may be a result of the inability of the cells to obtain sufficient calcium, in which case, as was previously stated,

the cells proceed to absorb enormous amounts of potassium. The calcium then accumulates in the root tissues of mottled citrus trees and the potassium tends to disappear.

Such absorption processes as this 'luxury consumption' of potassium that is brought about by toxic substances accumulating in citrus leaves may be looked upon as phases of parasitism within tissues of a tree induced by a chemical reagent such as boron. The cells of mottled leaves draw on the potassium of the roots more than they should and injury to the root system results. Toxic agents such as boron, deficient leaf calcium, excessive potassium absorption or resorption from root cells, and other additional concomitant salt effects not here discussed, eventually injure the cells of mottled leaves and these in turn injure the root cells. Amino acid, ammonia, nitrate, and carbohydrate studies that are being carried on should be more easily interpreted if the behavior of an element such as potassium, which may be intimately associated with carbohydrate or amino acid synthesis, is understood. Potassium is considered by many investigators as being associated with carbohydrate synthesis. Citrus leaves may deprive other tissues of the plant of a portion of their potassium supply and in such cases the leaves may become mottled. Leaf areas having deficient chlorophyll are ordinarily assumed to have a reduced carbohydrate synthesis. In severe cases of mottle-leaf there is a reduction in the commercial yield of fruit without an appreciable increase in the vegetative growth. *Pseudomonas radicicola* may absorb nitrate without synthesis of atmospheric nitrogen if the soil has nitrate available, and thus deprive the tissues of the host plant of this supply while still obtaining carbohydrates from the plant (Marshall[26]). As citrus leaves become old their requirement for potassium decreases very much below that when they are young. If citrus leaves mottle, then as they become older they appropriate supplies of potassium that should be available for root tissues.

Much of the calcium of the dry matter of citrus roots is insoluble in water. Unpublished results of Halma and Haas on the composition of the roots of leaf and leafy-twig cuttings grown in Hoagland's solution indicate that the soluble calcium in the dry matter as a percentage of the total ash ranges from about 1.47 to 4.68 per cent and the total calcium from about 7.00 to 22.00 per cent. Increasing the calcium nitrate in Hoagland's solution brought about increases in the total calcium, which could not be considered as due to culture solution adhering to the roots, because such adhering calcium should be water soluble when as a matter of fact the soluble calcium varied but little.

RELATION OF MANGANESE TO LOSS OF CHLOROPHYLL

Previous Work.—Bishop[2] has concluded, with most investigators, that manganese is essential for the growth of most plants but that toxicity results when manganese is present in excessive amounts. He associates manganese with chlorophyll formation, believing that low as well as high manganese causes loss of chlorophyll. The use of manganese was found to be very effective on tomatoes and other truck crops in calcareous Glade soil (Skinner and Ruprecht[38]). Many investigators have found applications of manganese effective against chlorosis: Allison, Bryan, and Hunter,[1] Willis,[44] Gilbert and McLean,[6] Schreiner and Dawson,[37] Lee and McHargue,[24] Miller,[30] and others. McHargue and Shedd[29] have reported the effect of manganese and other traces of elements on the growth of oats.

Samuel and Piper[36] have found gray-speck disease of oats to be a manganese-deficiency disease and as a result of this study have suggested that pecan rosette, mottle-leaf of citrus, and walnut yellows may possibly be manganese-deficiency diseases. They found the supplies of iron ordinarily used in culture solutions to contain manganese and therefore prepared their own iron salts.

The toxicity of large concentrations of manganese that brought about chlorosis in pineapple plants has been studied by McGeorge.[27] Jacobson and Swanback[20] have described the symptoms resulting from high manganese in tobacco. Some of the leaves were dwarfed and distorted, and in fully developed leaves the yellowish color was minutely distributed in the interspaces of the finest ramifications of the leaf veins, which long remained green. In later stages the entire leaves took on a more yellow color, but the pattern was unchanged, while in still later stages crinkling occurred and irregular brown spots were distributed over the leaf surface.

Manganese Deficiency and Mottle-Leaf.—Thus far in the writer's experiments the manganese contamination of the impure iron salts has either supplied manganese in amounts adequate for normal growth or else it is not an essential element in the growth of citrus. Investigations are being continued with regard to the essential nature of manganese for growth in citrus.

As will be shown later, high concentrations of manganese destroy the chlorophyll and bring about chlorosis. Experience has shown (Haas[11, 12] and Haas and Klotz[15, 16]) that with boron a deficiency and an excess are accompanied by entirely different symptoms in citrus;

TABLE 17

MANGANESE CONTENT OF HEALTHY AND MOTTLED ORANGE LEAVES

Variety	Date of collection of leaves	Location	Fertilizer treatment	Manganese in dry matter, in p.p.m.
			Healthy	
Navel	January 7, 1930	Plot U, Rubidoux	Manure, covercrop	22
Navel	January 7, 1930	Plot V, Rubidoux	Manure, covercrop	20
Navel	September 17, 1930	Plot K2A, Box Springs	Manure broadcast in spring	40
Valencia	May 22, 1929	Santa Fe, Calif.	Manure	34
Valencia	September 17, 1930	Plot K12, Box Springs	Manure trebled, broadcast in fall; covercrop	20
			Mottled	
Navel	January 7, 1930	Plot C, Rubidoux	Dried blood	29
Navel	January 7, 1930	Plot Q, Rubidoux	Nitrate of soda, blood, superphosphate, and sulfate of potash	28
Navel	January 7, 1930	Plot A, Rubidoux	Nitrate of soda, blood, bone, and sulfate of potash	41
Navel	January 7, 1930	Plot G, Rubidoux	Nitrate of soda, blood, and bone	23
Navel	January 7, 1930	Plot H, Rubidoux	Nitrate of soda	30
Navel	September 17, 1930	Plot K14, Box Springs	Nitrate of soda and covercrop	21
Navel	March 20, 1928	Hemet, Calif., along west road	Unknown	32
Valencia	January 23, 1930	Hemet, Calif.	Unknown	18
Valencia	September 17, 1930	Plot K28, Box Springs	Urea and covercrop	16

with manganese, however, as will be shown in a later publication, a deficiency as well as an excess is accompanied by chlorosis.

In order to determine whether mottled citrus leaves are deficient in manganese, as suggested by Samuel and Piper,[36] Washington Navel and Valencia orange leaf samples each consisting of 500 or more leaves were collected from healthy and mottled trees. Extreme care was taken in obtaining the samples to select only fully mature leaves. These were wiped free from dust and dried at 80° C. Ten-gram samples of finely ground dry matter were used in making the manganese determinations. The method employed for the determination of manganese was that described by Samuel and Piper,[36] which proved most satisfactory.

Table 17 gives the results obtained. It is at once seen that mottled leaves are not suffering from a deficiency of total manganese. The soluble manganese has not as yet been determined. These results indicate the extreme hazard of single determinations of healthy and mottled leaves because the values for the manganese content of both types of leaves extend over the same, but a rather wide, range. A larger number of leaf samples may be necessary before it can be determined conclusively whether an excess of manganese is associated with mottle-leaf. The final test, however, in determining whether a deficiency of manganese is associated with mottle-leaf must be to attempt to grow citrus in the complete absence of manganese. This has been done and the results will be reported in a later publication.

Effect of High Concentrations of Manganese on Valencia Orange Leaves.—In the present studies about forty young budded trees were grown in sand cultures in containers (20 inches in diameter and 24 inches deep) and in 12-gallon earthenware crocks. The trees represented in the cultures were lemon, orange, grapefruit, and avocado. The cultures received Hoagland's solution during the first year and then this solution to which 200 p.p.m. of manganese was added in the form of $MnSO_4 \cdot 2H_2O$. When injury was apparent, the concentration of manganese was reduced to 100 p.p.m., but the new growth was far from being healthy.

When manganese-affected Valencia orange leaves were collected and their content of manganese determined, it was found that the dry matter contained 680 p.p.m. of manganese. This is about 30 times that normally found in citrus leaves.

In some cases the manganese caused the occurrence of small dot-like burned spots on both surfaces of young Valencia orange leaves (fig. 26). These spots did not spread and did not prevent the leaves from becoming dark green and from reaching maturity.

Fig. 26. Dry, sunken spots on both surfaces of Valencia orange leaves on trees grown in sand cultures rich in manganese.

Fig. 27. Valencia orange leaves from trees in sand cultures that received high manganese. Upper row: dorsal surface showing destruction of chlorophyll and gum formation; lower row: leaf to the extreme left (dorsal surface) with dry, sunken spots as in figure 26, four leaves to the right (ventral surface) showing burning of young chlorotic leaves under extreme conditions.

Young Valencia orange leaves under extreme conditions of manganese toxicity became chlorotic and burned on the ventral surface

Fig. 28. Manganese effects on mature Valencia orange leaves from trees in sand culture. Upper row: dorsal surface showing a type of mottle in which gum deposits eventually make their appearance. Lower row: ventral surface showing minute raised gum deposits which may merge as they become very numerous.

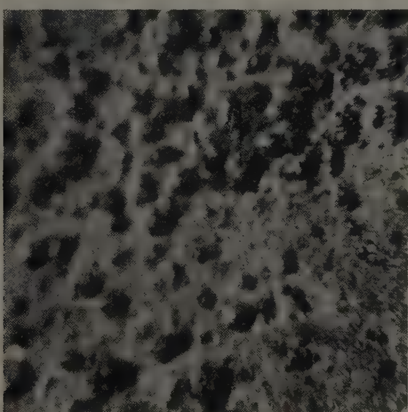

Fig. 29. Close-up view of resin spots on ventral surface of Valencia orange leaves collected from trees injured with excessive manganese in sand cultures.

as seen in figure 27. Leaves affected with an excess of manganese adhered to the twigs for a long period unless entirely chlorotic or burned, in which case abscission occurred prematurely.

At certain stages of injury the manganese-affected Valencia orange leaves showed a type of mottle when seen from the dorsal surface. As the injury progressed the mottled areas became spotted with gum deposits as may be seen in figure 28. This type of mottling presented

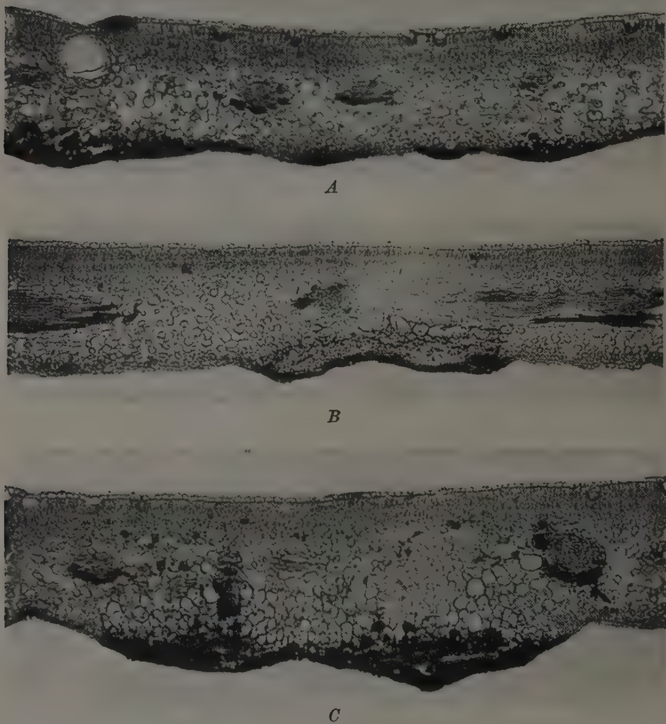

Fig. 30. Cross sections of resin spots on the ventral surface of Valencia orange leaves grown in sand cultures that received excessive manganese. *A*, Undulating appearance of ventral surface as a result of numerous resin spots; *B*, cell proliferation and resin between the cells of the deeper affected region; *C*, resin-filled cells near the ventral surface with resin between or at the surface of the deeper lying cells.

a very different appearance from that commonly found in groves of southern California. The ventral surface of such leaves became covered with numerous minute rusty-brown deposits of resin which may merge as they increase in number. This spotting was very characteristic of orange leaves affected with excessive manganese.

The nature of the minute resin spots on the ventral surface of Valencia orange leaves may be of interest. If figure 29 is examined, it may be seen that they are raised and very numerous.

A better understanding of the nature of these spots on Valencia orange leaves may be had from a study of a cross section of such a spotted leaf. Figure 30*A* shows the undulating effect given the ventral leaf surface because of these spots. The resin appears in *B* and *C* to occur along the cell wall or between the cells except in the raised portion where the cells themselves appear to be filled with gum or

Fig. 31. Avocado leaves showing the effects of excessive manganese when grown in sand culture. Two green leaves on the left show russet-brown stain on both sides of the veins. No obvious gum deposits. The two leaves at the right are mature leaves, yellowish in color and ready for abscission; the leaf at extreme right is from a control tree and does not show the deep coloration along the veins.

resin. Reed and Haas[35] have described cell proliferations occurring from the dorsal surface of the palisade tissue in orange leaves. In the present case where resin spots also occur on the dorsal leaf surface, it is very likely that such palisade cell proliferation may occur here.

Effect of High Concentrations of Manganese on Avocados, Lemons, and Grapefruit.—The effect of manganese on the leaves of avocado trees in sand cultures is seen in figure 31. There is a brownish coloration (with no obvious gum along the veins) characteristic of manganese. This is in striking contrast with the effects produced by many other salts that cause burning at the tip and leaf margins or between the veins.

Fig. 32. Effect of high concentrations of manganese upon leaves of lemon trees in sand cultures: upper row, dorsal surface; lower row, ventral surface. Gum deposits occur in the chlorotic areas.

Fig. 33. Grapefruit leaves collected from trees in sand cultures that received an excess of manganese. They show large, dry spots on the dorsal surface. Young leaves may be deformed as in the upper row (second and third leaves from the left).

Figure 32 shows the type of mottle that occurred in lemon leaves and the gum or resin spots that are most pronounced on the dorsal surface. In some cases the mottle became a true chlorosis. The type of mottle is very different from that commonly found on lemon trees in the citrus districts of southern California. In very late stages a burning of the chlorotic areas may occur.

Grapefruit leaves from trees in sand cultures frequently showed, as a result of excess manganese, large, brown, burned spots with yellowish-green areas surrounding them, as seen in figure 33. Such leaves did not burn along the tip or margin. Young leaves were sometimes crinkled and curved laterally, causing considerable deformity which persisted until the leaves were ready for abscission.

Grapefruit leaves sometimes showed a destruction of the chlorophyll between the veins on their dorsal surface (upper row, fig. 34).

Fig. 34. Grapefruit leaves from trees in sand cultures showing symptoms as a result of excessive manganese. Upper row: a type of mottle on dorsal surface with burning or gumming in the mottled areas. Lower row: ventral surface showing numerous minute resin spots that frequently are clustered together. Dorsal and ventral spots frequently unite.

Burning or gumming occurred in these mottled areas, which gives the leaves a different appearance from the mottle-leaf commonly seen in the groves of southern California. The ventral surfaces of the lower row of leaves in figure 34 were covered with numerous rusty brown-resin spots, which sometimes occurred in clusters. Even though these spots were often very numerous, the leaf tip or margin did not burn.

In severe cases the resin spot on one side of the leaf was continuous so as to show also on the other side. This was likewise true in the case of orange leaves.

When the manganese was omitted from the solution that was given the sand cultures, some of the previously affected leaves were observed

Fig. 35. Grapefruit leaves collected from manganese-affected trees in sand culture after the new growth on the trees began showing improvement after the omission of the manganese.

as the new portions of the tree showed improvement. Figure 35 shows the dorsal surface of several leaves in which the spots took on a concentric form and in some cases became thickened and raised. A yellow area usually surrounded each spot. The absence of tipburn is characteristic in manganese injury.

SUMMARY

The technique for growing citrus cuttings for several years in water cultures containing boron and zinc is described. Large concentrations of zinc injured the roots and dwarfed the tops of lemon seedlings by shortening the stem axis and reducing the leaf size, giving the tops a rosette-like appearance.

Beryllium, like lithium and boron, is extremely toxic in small concentrations to citrus cuttings in water culture. Lemon leaves affected with an excess of beryllium show a type of mottle that may approach variegation. These three elements have the lowest atomic weights of the nongaseous elements in groups II, I, and III respectively, and although all are very toxic at relatively low concentrations, their toxic effects upon citrus are dissimilar.

Another type of mottling has been produced upon citrus seedlings in culture solutions containing picric acid.

Urea-containing compounds when used in excess on citrus in soil have caused yellowing of the leaf tips, or a mottle near the apex of the leaves. Ammonium compounds in excess are toxic to citrus, especially when the nitrate supply is low.

When citrus leaves have been given a deficient nitrogen supply for an extended period and are then given nitrogen, some portions of the leaf blade may become dark green while others may remain yellow without burning.

Excessive concentrations of calcium nitrate were noninjurious to Valencia orange trees in artificial soil (Sierra loam) cultures with excellent drainage. The use of abundant water rich in nitric acid in such cultures gave healthy growth. Sodium nitrate in large amounts in such soil cultures was less injurious when drainage was not impaired. The extent of the injury in the citrus trees varied according to the concentration of the sodium nitrate used and the extent of drainage.

Differences in the growth of citrus trees on some of the Rubidoux fertilizer plots of the Citrus Experiment Station were reflected in the growth of St. Michael orange seedlings grown (in artificial cultures) in soil from such plots. The relation of the composition of green manure crops to the fertilizer treatment was studied.

The leaching of Sierra loam soil cultures with weak sulfuric or phosphoric acid brought about gum formation in orange leaves.

Chromates added to soil cultures were very toxic to citrus, but chromium cations added to soil cultures were noninjurious, possibly as a result of precipitation.

The continued leaching of Sierra loam soil cultures with weak Hoagland's solution lacking calcium and saturated with carbon dioxide brought about premature leaf abscission of Florida orange seedlings, largely as a consequence of the loss of soil calcium and the abundant supply of potassium.

Chlorotic citrus leaves when sprayed with iron-containing solutions show a dark green spot where each drop of solution concentrates as the water evaporates. It is very significant that mature chlorotic citrus leaves have the same composition as mature mottled leaves. There is a close relation in certain cases between mottle-leaf and chlorosis. The use of 20 p.p.m. of copper sulfate in the culture solution of Valencia orange trees in sand, gave the leaves a mosaic appearance as a result of the intimate mixture of the small green and yellow irregular areas of chlorophyll.

Excessive chloride is very injurious to citrus in sand cultures even when calcium is the prevailing cation.

In the absence of soluble calcium (other than that obtainable from finely powdered calcium carbonate), Valencia orange trees in sand cultures that received Hoagland's solution lacking calcium were injured much more when potassium was substituted for the calcium in Hoagland's solution than when sodium was substituted. These cultures show the extremely large absorption of potassium when soluble calcium is deficient. In the absence of soluble calcium, citrus in sand or solution cultures absorbed large amounts of sodium or potassium according to which was present, but the absorption of potassium was the greater of the two. A deficiency of soluble calcium may exert a tremendous effect upon the absorption process and thereby upon the health of citrus.

Higher percentages of sodium and potassium and lower percentages of calcium were found in mottled than in healthy citrus leaves. The soluble calcium as a percentage of the soluble ash is roughly constant for healthy mature citrus leaves and is much less in mottled than in healthy leaves. The insoluble calcium as a percentage of the insoluble ash is roughly constant regardless of whether the leaves are mottled or healthy. The maintenance of a percentage of soluble calcium (within a given range) is paramount for health in citrus leaves.

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